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# Assessment of Future Environmental Trends and Problems: Industrial Use of Applied Genetics and Biotechnologies

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# ASSESSMENT OF FUTURE ENVIRONMENTAL TRENDS AND PROBLEMS: INDUSTRIAL USE OF APPLIED GENETICS AND BIOTECHNOLOGIES

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16. ABSTRACT

The proposed study is to be a technological assessment of genetic engineering as it applies to commercial industries and its potential effects on the environment. This includes a detailed literature review and state of the art analysis of genetic engineering, an analysis of how applied genetics will affect public health and public welfare, its probable impact on the environment and environmental policies and an analysis of knowledge gaps, including identification of inadequacies of analytical methods and techniques. Additionally, the socioeconomic impact of genetic engineering on commercial industry will be examined.

The approach will include a literature review of five key industrial sectors: Pharmaceutical and Cosmetic, Industrial Chemical, Energy, Food Manufacturing and Preservation, and Mining. Areas that will be examined at length include: Environment and Populations, Government Policy, and Technology.

The research will be carried out in three phases:
I Development of Data Base
II Potential Hazards of Genetic Engineering
III Analysis of Findings

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# ABSTRACT

This study represents a portion of an overall EPA/ORD assessment of future environmental trends and problems. With the aim of providing the EPA with information on emerging technologies, the focus of this report is the industrial use of applied genetics. The following five industrial sectors are examined: pharmaceutical, chemicals, energy, mining, and pollution control.

Following a brief historical review of the important developments in basic biological research that heralded the advent of modern biotechnology, the report describes the variety of experimental and commercial techniques that are encountered in this field. These various methods include recombinant DNA technology, mutagenesis, cell fusion procedures, immobilized bioprocesses, and fermentation technology.

In a section entitled Interested Parties, the report lists the numerous individuals and organizations that are actively involved in the commericalization of applied genetics. Over 100 U.S. business firms and about 50 foreign concerns are identified as having substantial commercial interest in biotechnology. This section also describes the role of various U.S. government agencies in examining progress in this field.

The bulk of the report consists of an industry-by-industry analysis of current R&D activities in biotechnology, an estimate of future prospects, and an assessment of potential environmental and health hazards associated with these activities. Trends are identified and, wherever possible, schedules for the appearance of new applications are predicted.

The final section of the report summarizes the findings and provides a number of recommendations to the EPA regarding future action in the field of applied genetics.

This report is submitted in fulfillment of Contract No. 68-02-3638 by Teknekron Research, Inc. under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period October 1, 1980, to February 28, 1981, and the work was completed as of March 31, 1981.

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# SECTION I

# INTRODUCTION

## 1.1 Scope of the report

The report is organized into five sections. Sections 1 and 2 provide, respectively, historical background for and a description of the techniques utilized by the applied genetics industry. Section 3, entitled Interested Parties, provides a listing of academic, government, and commercial concerns that have a stake in the applied genetics industry. Both foreign and domestic concerns are included. In Section 4 we present a detailed account of the various projects and activities currently underway or planned within the applied genetics industry, and we also identify and assess the environmental and health hazards posed by this new industry. The information in Section 4 is presented according to industrial sector. The following industries are examined:

- pharmaceuticals
- industrial chemicals
- energy
- mining
- pollution and waste management

Section 5 summarizes our general understanding of the sources and magnitudes of the potential adverse environmental and health effects of applied genetics. Questions requiring further study are identified, as are possible areas of future concern to the EPA.

## 1.2 Brief history

The purposeful manipulation of hereditary information in plants and animals by humans, as well as the exploitation of microbial processes, has occurred since the time mankind formed societies. An understanding of the **biological** nature of these processes has been acquired only recently (i.e., during the past 100 years), and their **chemical** basis has been unravelled even more recently (in the past 35 years). A variety of terms are now employed to encompass this field of knowledge, including **applied genetics, biotechnology, bioengineering,** and **genetic engineering.** While recognizing that these general terms connote subtle differences in scope, we will use them interchangeably in this report. However, certain bioengineering procedures, such as **recombinant DNA** technology, entail specific activities that require more careful definition. These techniques are described in detail in Section 2 of the report.

Examples of genetic practices and microbial processes that have ancient origin include the following: alcohol fermentation, cheese production, food crop and domestic animal breeding, crop rotation, and the use of human and animal wastes as fertilizers. The utility of animal and plant breeding and selection was long ago recognized as a controlled method of generating improved strains of vital food crops and hardier domesticated animals. This ancient realization likely arose from the observation that children tended to possess various features characteristic of each of the parents, although the reasons for these similarities were unkown. Alcohol and cheese fermentations were undertaken long before the microbial basis for these processes was recognized. Likewise, occasional planting of fields with leguminous crops, such as soybeans, peas and alfalfa, proved to be a helpful, often crucial, means of replenishing spent soil before it became known that bacteria were responsible for this outcome by virtue of their ability to convert atmospheric nitrogen into usable, chemically reduced forms, such as ammonia. This is the process of nitrogen fixation. And, lastly, ignorance of the role of soil bacteria in recycling human and animal solid wastes did not prevent ancient cultures from employing this rich source of nutrients to improve crop production.

The biological basis of these various processes was recognized beginning in the latter half of the nineteenth century. Two separate findings were essential to the genesis of this understanding. First, during the years 1856 to 1868, an Austrian monk named Gregor Mendel demonstrated in his experiments with peas that numerous observable traits, such as flower and seed colors, are passed from parent to offspring in the form of discrete units of heredity and that each parent supplied independent traits. These revolutionary findings, which were ignored by the scientific community until early in the twentieth century, provide the basis for the **gene theory of inheritance**, which states that the multitude of traits that constitute an individual organism are expressions of discrete hereditary units, called **genes.** In higher organisms, these genes are located on **chromosomes** within the **nucleus** of each cell. In lower forms of life, such as bacteria, which lack a defined nucleus, the chromosomes nevertheless consist of genes. In all life forms, genes provide the information that determines the make-up of the organism itself, as well as the means whereby traits are extended to the next generation.

The second fundamental discovery that led to an understanding of the biological nature of ancient endeavors in the realm of applied genetics was that of Louis Pasteur. In 1860, he demonstrated that alcohol production from fermentable substrates depended on the presence of viable microorganisms called yeasts. This finding provided the initial example of a living microbe performing a commercially useful process. Today's genetic engineering industry holds the promise that many thousands of commercially useful products and processes will result from applications of recent discoveries in biology that owe their heritage in part to the findings of Mendel and Pasteur.

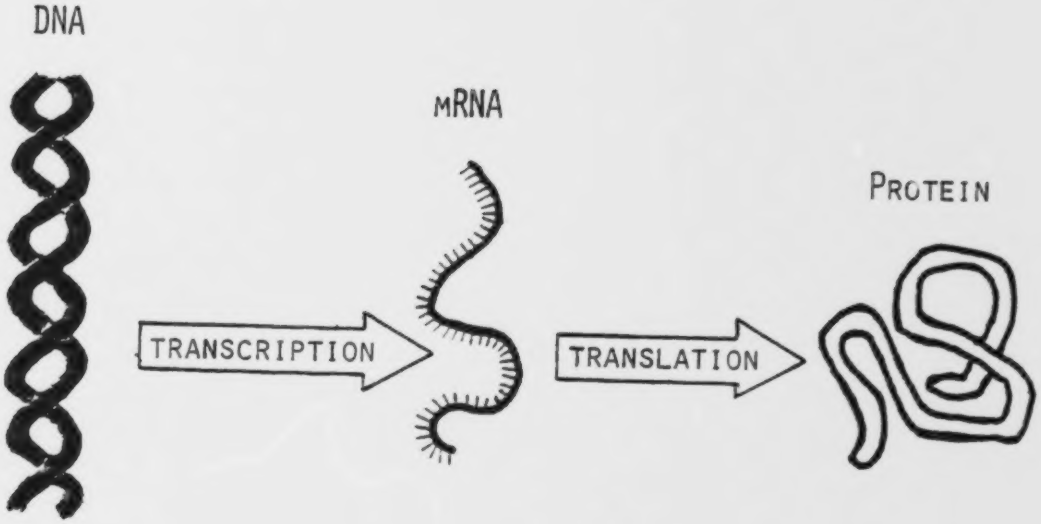
The **chemical** basis of genetics was uncovered only recently. Although DNA (deoxyribonucleic acid) was located in cell nuclei in 1869, its role as the bearer of genetic information was not revealed until 1944 by Oswald Avery and co-workers. They demonstrated that pure DNA isolated from virulent pneumococci bacteria was absorbed by a non-virulent pneumonia strain and thereupon transformed to the virulent form. Further substantiation of the genetic function of DNA was provided in 1952 by A.D. Hershey and M. Chase, who radioactively labelled both protein and DNA constituents of bacteriophage viruses. (Bacteriophage are simple viruses that infect bacteria; they consist solely of a protein coat surrounding a DNA core.) Infection of susceptible bacteria by these radiolabeled viruses resulted in the finding that viral DNA is necessary and sufficent to mediate the infection. Viral protein is not required.

The above-mentioned studies confirmed the role of DNA as the bearer of genetic information in living systems. It is now well-established that DNA alone serves this purpose in all forms of life, both plants and animals, both primitive and advanced. The information contained within the chemical structure of DNA determines to the full extent the biological nature of the organism (i.e., its appearance and its life functions). (The only exception to the universality of DNA as the genetic material is certain viruses, called retroviruses, that employ ribonucleic acid, or RNA, in this role. Although they constitute an exceedingly small proportion of the total biota on the planet, these viruses are important because they induce malignant tumors in mammals including, probably, humans. Even so, retrovirus RNA is copied into DNA during the process of infection.)

Knowledge of the chemical means whereby DNA maintains and replicates the cell's store of genetic information evolved during the 1950's and 1960's. Many scientific investigators contibuted during this time to this advance in understanding, but several steps in particular bear mentioning. In 1953, James D. Watson and Francis Crick proposed a double-helical structure for DNA. This model readily suggested a mechanism whereby DNA could be faithfully reproduced. During the mid-1960's, Arthur Kornberg and associates worked out many of the biochemical details of this replication process. Meanwhile, the genetic code was being broken, most notably by Marshall Nirenberg and colleagues. This code determines how the sequence of chemical constituents in DNA is translated into a specific sequence of amino acids (via a nucleic acid intermediate called messenger RNA, or mRNA). Amino acids are the chemical building blocks of proteins which, in turn, provide structural integrity and mediate metabolic acitivites within every cell of every organism. The steps in the pathway from DNA to protein are diagrammed in Figure 1-1.

This basic research in molecular biology and genetics paved the way for developments during the 1970's that have given rise to the technology of recombinant DNA. These later achievements and procedures will be detailed in Section 2 of this report. It must be recognized that the modern field of applied genetics, with all its promise for future benefits to mankind (and its potential dangers), could not exist today but for the numerous accomplishments in basic research in biology and biochemistry over the past several decades, only a few of which are mentioned above.

Figure I-1
Steps in the process of gene expression


DNA
mRNA
PROTEIN
TRANSCRIPTION
TRANSLATION

# SECTION 2

# TECHNOLOGY OF APPLIED GENETICS

Applied genetics as practiced by ancient societies involved a minimum of human intervention and consisted of little more than allowing nature to take its course. Thus, alcohol and cheese fermentation and the recycling of wastes, processes that we now know to be mediated by microorganisms, were undertaken merely by exposing the appropriate raw materials to the environment, whereupon a transformation of the substrates took place. Controlled animal and plant breeding was implemented by placing prospective parents in proximity to one another. Ancient bioengineering technology, therefore, succeeded by virtue of man's ability to manipulate crudely the **biology** of his environment.

By contrast, the emergence of modern biotechnology as a scientific discipline that holds enormous potential for benefiting mankind stems from our recently acquired ability to comprehend and manipulate the **chemistry** of living systems. Thus, the currently popular notion that modern society is embarking on the "Age of Biology" could be slightly rephrased to become the "Age of Biochemistry."

The modern technology of applied genetics encompasses a variety of procedures and processes. Each of these will be dealt with separately in the remainder of this section.

## 2.1 Recombinant DNA techniques

Recombinant DNA technology refers to the ability to isolate fragments of DNA from separate sources and to splice them together chemically into a functional unit. The DNA fragments can derive from the same organism or from different organisms in the same species (techniques that have considerable future potential for gene therapy applications in humans), but the currently most promising technique involves the joining of DNA segments from disparate species of organism, such as bacteria and humans. This latter approach has been utilized, for example, in recent efforts to mass-produce human interferon, a drug that may combat viral diseases and cancer.

A review of the recent developments in molecular biology that have led to the emergence of recombinant DNA technology can best be presented by considering those specific laboratory procedures necessary to carry out such experiments. There exist six distinct phases in the process.

### (1) Isolation and purification of DNA

Since DNA exists naturally as a long, fragile, chain-like structure, techniques for gently isolating extended sequences containing intact genes were needed. Such procedures, which include high-speed centrifugation and electrophoresis, were developed during the early 1960's, largely by Julius Marmur and colleagues.

### (2) Fragmentation of DNA into reassociable segments

This crucial step is mediated by a class of bacterial enzymes, called **restriction endonucleases,** that introduce widely spaced breaks at specific sites in the DNA chain. The nature of the cuts is such that the separated ends (so-called "sticky ends") can readily reassociate with one another, thereby regenerating the original cleavage site. The rejoining can involve two DNA segments that each derive from different sources, so long as the DNA from each source was clipped into fragments by the same restriction endonuclease. Discovery of these enzymes and elucidation of their physiological role are largely credited to Werner Arber in Switzerland and to Dan Nathans and Hamilton Smith at Johns Hopkins.

### (3) Sealing DNA fragments together

The rejoining of DNA fragments by way of their sticky ends requires a further step for the full stabilization of the recombined unit. Another enzyme, called polynucleotide ligase or simply **ligase**, performs this function. The ligase enzymes were discovered independently by a number of investigators, including Malcolm Gefter at MIT and Arthur Kornberg at Stanford.

### (4) Replication and maintenance of recombinant DNA molecules

Once DNA fragments have been cut-and-spliced together *in vitro*, a suitable **host** organism must be found into which the recombinant DNA can be stably incorporated and reproduced. The enteric bacterium, *Eschericia coli*, (or *E. coli*), was the obvious first choice as a host since more is known about the genetics and molecular biology of this microbe than of any other organism. The replication of a DNA segment by *E. coli* requires that the segment contain a specific short sequence of DNA that serves as a signal to the enzymatic machinery inside the cell. This signal, sometimes called the **origin of replication**, can be found on certain small, self-replicating loops of DNA, called **plasmids**, that are commonly found inside bacterial cells. (Plasmids reproduce themselves independently of the major chromosome in bacteria and they are readily transferred between different bacterial strains. In addition to other functions, plasmids are responsible for the resistance to numerous antibiotics that has become a major medical problem in recent years.) Thus, incorporation *in vitro* of the recombinant DNA molecule into a bacterial plasmid, followed by reintroduction of the hybrid plasmid into the bacterial cell, will permit stable replication of the recombinant DNA.

Alternatively, if the recombinant DNA could be incorporated into the major chromosome of the host bacterium, then it would be replicated as part of that chromosome. This is possible through the use of a particular bacteriophage, called **lambda**, that infects *E. coli*. Upon infection, lambda DNA becomes incorporated into the bacterial chromosome where it replicates along with the host chromosome. Thus, attachment of the recombinant DNA molecule to lambda DNA prior to the infection of E. coli will similarly allow replication of the recombinant DNA.

Both plasmids and lambda bacteriophage are termed **vectors** owing to this ability to transfer recombinant DNA into suitable hosts for replication. A number of scientists pioneered the effort to demonstrate the usefulness of vectors in gaining expression of exogenous or foreign DNA in E. coli, including Stanley Cohen and Paul Berg at Stanford, and Herb Boyer at the University of California, San Francisco.

There exists a direct method of putting foreign DNA into host bacteria without the need for intact viruses or plasmids. Pure, naked DNA can be absorbed by bacterial cells in a process called **transformation.** This is the procedure used by Avery and co-workers in 1944 to "transform" non-virulent pneumococcus strains into virulent bacteria. Some bacterial strains, including E. coli, must undergo a simple chemical pre-treatment with calcium salts in order to make them amenable to DNA uptake.

**(5) Selection of cells containing recombinant DNA**

Since only a small percentage of potential host bacteria do in fact acquire recombinant DNA by way of these procedures, it is necessary to perform a selection step. Depending on the type of vector used, it is possible to screen for antibiotic resistance (when the vector is a plasmid containing an antibiotic resistance gene) or to screen for the presence of viable bacteriophage viruses (when lambda is used as the vector). These selection methods give rise to **clones** of bacterial hosts containing recombinant DNA; that is, each bacterium in the clone is derived from a single progenitor cell that multiplied repeatedly, with exact copies of the cell's DNA having been distributed into each daughter cell. The segment of recombinant DNA contained therein is also replicated; that is, it has been cloned.

### (6) Expression of recombinant DNA into gene products (proteins)

The recently acquired ability to incorporate exogenous DNA into bacteria, and to have that DNA replicated as though part of the bacterial genetic complement, is of considerable scientific interest. But commercial applications of this new technology demand that foreign genes implanted into bacteria be expressed into the proteins encoded by that DNA. For example, in order to convert E. coli into "factories" capable of producing human insulin, it is necessary both that the gene for insulin is stably maintained in the bacteria and that the human DNA segment is transcribed into messenger RNA, then translated into insulin (see Figure 1.1). As mentioned above, gene replication (maintenance) is assured by the presence of certain genetic signals. Similarly, the processes of **transcription** and **translation** rely on signals that inform the cell's enzymatic machinery where to start and where to terminate each of these processes. All of these various signals must be present at the appropriate locations in the DNA in order for gene expression by recombinant DNA methodology to be successful.

Once a bacterial cell has been "tricked" into manufacturing a human or other foreign protein, additional problems arise. The bacterium may recognize insulin as a "foreign" protein and may degrade it before it can be recovered. If stable, the foreign protein may simply accumulate inside the bacterial cell, necessitating its recovery by breaking open the cells--a tedious and inefficient process. Ideally, the foreign protein will be excreted out of the host cell into the growth medium from which it can be readily purified. Clever techniques are now available to bring this about, and improvements are being made continuously.

One additional roadblock bears mentioning. Many human proteins possess attachments that consist of sugar molecules. These **glycoproteins** are especially common in blood serum; e.g., interferon is a glycoprotein, although insulin is not. Bacteria do not possess the machinery to synthesize or attach sugars to proteins. Although the precise function of the sugars in unclear, it is probable that they serve a useful, perhaps crucial, role in maintaining the physiological activity of the protein. Thus, considerable effort is underway to develop microbial host organisms that can attach sugars to proteins. Common brewer's yeast, or

Saccharomyces cerevisiae, is likely to be the preferred host cell for this purpose. Although it is a single-celled microbe, yeasts belong to the general class of higher organisms that include humans, namely **eukaryotes.** Eukaryotic organisms are classified on the basis of their having a nuclear membrane surrounding the genetic material within each cell. Bacteria and certain algae, on the other hand, compose the class of organisms called **prokaryotes** (i.e., those lacking a defined nuclear membrane). Although researchers in recombinant DNA have predominately utilized E. coli as the host organism, there is no doubt that the future commercial success of the technology hinges on the increasing use of eukaryotic hosts such as yeasts and fungi.

A general scheme showing the steps involved in a recombinant DNA experiment is diagrammed in Figure 2-1.

### 2.2 Genetic alterations induced by non-recombinant DNA procedures

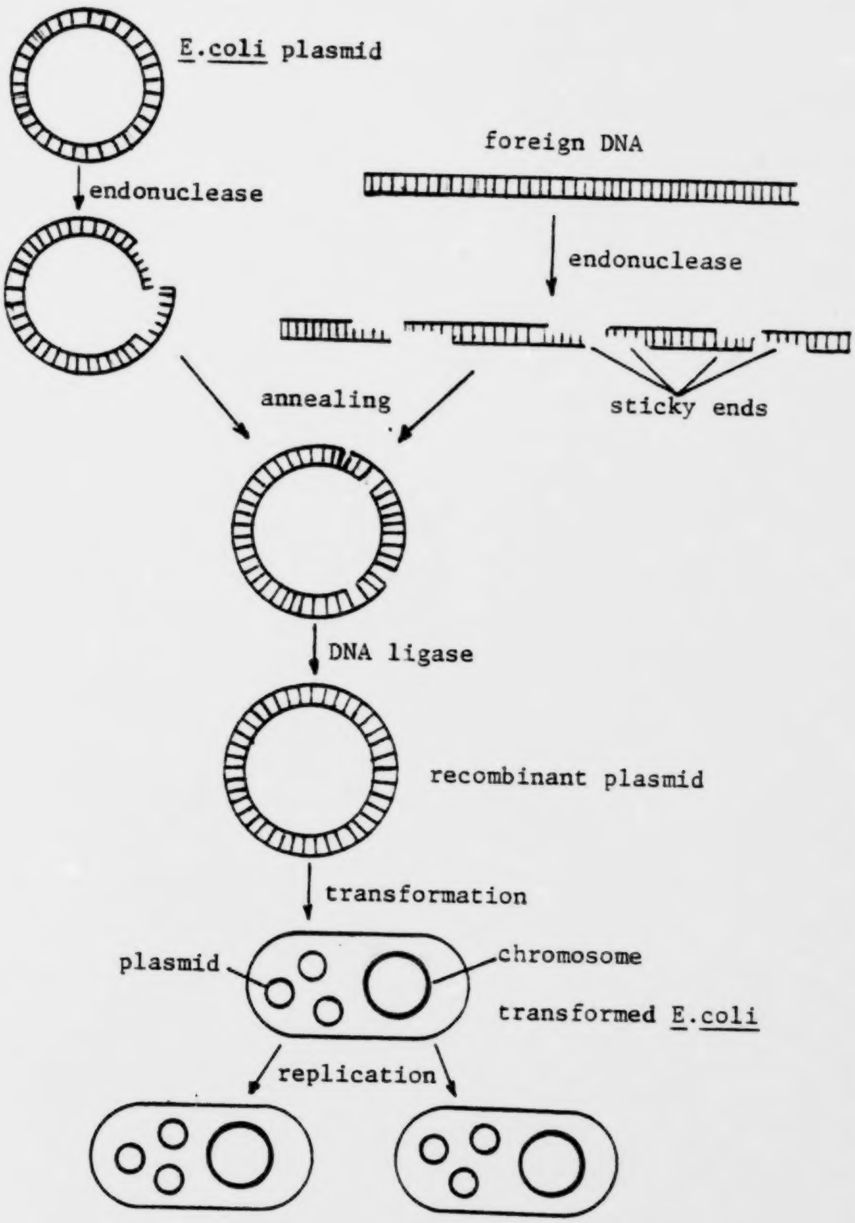
A number of techniques are currently utilized to induce genetic alterations in cells. The technology of recombinant DNA represents the most recently developed and the most glamorous of these procedures, and it holds the powerful advantage that the outcome of these alterations can be predicted and controlled to a greater extent than with other techniques. Nevertheless, other gene-altering procedures are available, several of which had been in use for many years prior to the advent of recombinant DNA technology. These alternative methods will be described next.

#### 2.2.1 Induced mutations (mutagenesis)

The DNA of all living cells is continuously undergoing slight changes in its composition as a consequence of interacting with its external environment. These alterations, called **mutations,** are thought to be the driving force for the

Figure 2-1

Generalized scheme depicting the steps in conducting a recombinant DNA experiment


E.coli plasmid
foreign DNA
endonuclease
endonuclease
annealing
sticky ends
DNA ligase
recombinant plasmid
transformation
plasmid
chromosome
transformed E.coli
replication

evolution of organisms into new species and, under natural conditions, they occur at a low rate. Under experimental conditions, however, agents that induce genetic mutations, called **mutagens,** can be administered in order to accelerate greatly the rate of mutation. This is the process of **mutagenesis.**

A variety of mutagens are used experimentally and commercially to induce mutations. In general, mutagenic agents operate by interfering with the normal cellular processes involved in the repair of DNA. (Healthy cells maintain this enzymatic system for fixing mutations that arise from naturally occurring sources.) Ultraviolet (UV) radiation and chemical agents such as nitrosoguanidine and acridine are the most commonly used mutagens.

The induction of mutations by methods such as these is highly non-specific; that is, the experimenter cannot control the genetic site at which the mutation will occur. Therefore, following the mutagenic step, it is necessary to conduct a **selection** for those mutated organisms that possess the desired traits. For this reason, commercial mutagenesis is feasible only with organisms that have a relatively short **generation time,** such as microorganisms. Since a single bacterial cell will grow to a visible colony within a few days, it is possible to observe the effect of the mutagenic procedure in short order. Moreover, many thousands of such colonies can be screened simultaneously. Nevertheless, a mutagenic procedure was recently described involving **plant** cells growing in tissue culture. This advance suggests that genetic alterations in plants generally will become feasible by way of induced mutations.

Mutagenesis methods have found widespread utility in the pharmaceutical industry to enhance production of substances from microbial sources. Particular success has been achieved with the antibiotic penicillin, which derives from a strain of mold, and gentamycin, which is produced by a bacterium of the Streptomyces species. Unlike recombinant DNA methods, mutagenesis is incapable of endowing the microbe with properties that it does not already possess.

That is, no new genetic material is introduced; rather, existing capabilities are enhanced.

### 2.2.2 Cell fusion methods

A method whereby one cell type can be endowed with properties of another cell involves fusing those two cells together into a single unit. This procedure is now commonplace in the experimental laboratory and has been applied to a variety of cells from microbes to man and from both plants and animals. The methodology is relatively inexpensive and is essentially the same regardless of the cell type involved. Two technical approaches are in general use.

#### (1) Monoclonal antibodies (hybridomas)

Mammals have evolved a complex internal system of defense against foreign intruders, such as bacteria and viruses. This **immune system** functions in part by producing proteins called immunoglobulins, or antibodies, that specifically recognize and eliminate these alien invaders. A typical immune response to a bacterium, for example, consists of a great variety of different antibody molecules, each capable of recognizing and binding to a specific **antigen** on the surface of the microbe. Each of these distinct antibody types is manufactured by a **clone** of antibody-producing cells. These cells are called **lymphocytes** and since numerous clones of lymphocytes are each reacting to the presence of the bacterium, the response is termed **polyclonal.**

Antibody preparations (antisera) have long been used to great advantage as diagnostic agents. **Immunodiagnostic assays** currently comprise approximately one-fourth of all tests performed in the clinical laboratory. Such assays are helpful in rapidly diagnosing bacterial or viral infections and in monitoring drug or hormone levels in blood and urine.

From a physiological standpoint, polyclonal antibody responses to antigens are highly advantageous since they ensure that the individual will effectively repel foreign invaders. But to the clinical chemist, the diversity of antibodies can be bothersome since closely related antigens may not be distinguishable using these conventional antisera. In 1975, a technique was described by Cesar Milstein in Cambridge, England, that permits the generation of **monoclonal** antibodies; that is, immunoglobulins derived from a single cellular source or a single clone of cells. Such antibody molecules are all chemically equivalent to one another. The technique simply involves mixing antibody-producing cells (lymphocytes) with cells from a type of tumor, called a **myeloma**, that are themselves derived from lymphocytes. A fusing agent is added that partially dissolves the membrane that surrounds both cell types, thereby permitting contiguous cells to merge together. A common organic polymer, polyethylene glycol, serves as a satisfactory fusing agent. After removal of the fusing substance, the fused cells are grown in **tissue culture** (see below) and desired clones are identified by a suitable selection procedure. Such a clone combines the qualities of the two contributing cell types: it secretes a specific, monoclonal antibody and it grows continuously and rapidly owing to its tumor-like properties. This dual capability is reflected in the term **hybridoma**, which is applied to a clone of cells secreting monoclonal antibodies. A diagram of the steps involved in generating hybridomas is shown in Figure 2-2.

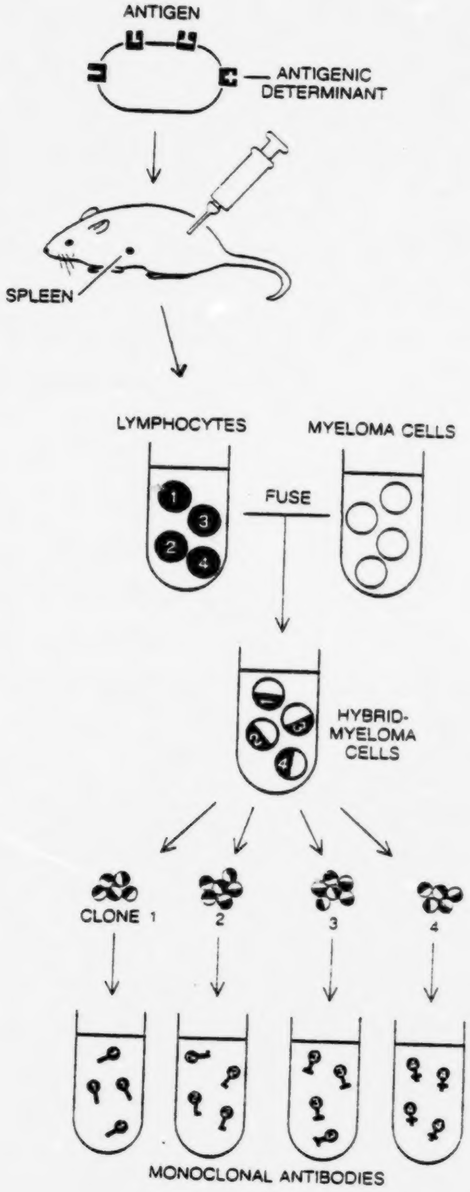
The initial demonstration of the hybridoma technique and subsequent commercialization of the process have involved cells derived from laboratory mice. The technology was recently extended to the use of human lymphocytes. This advance will soon lead to monoclonal antibodies for in vivo diagnostic and therapeutic uses.

### (2) Protoplast fusions and plant tissue culture

The second general class of fusion techniques involves microorganisms or plant cells. These cell types possess a rigid, protective **cell wall** that surrounds the

Figure 2-2

Steps in the process of generating monoclonal antibodies


ANTIGEN
ANTIGENIC DETERMINANT
SPLEEN
LYMPHOCYTES
MYELOMA CELLS
FUSE
1
2
3
4
HYBRID-MYELOMA CELLS
CLONE 1
2
3
4
MONOCLONAL ANTIBODIES

Source: Milstein, C. (1980)

cell membrane. (Animal cells lack cell walls.) The wall from such cells can be readily removed using enzymes that specifically digest the cellulose-like substance that comprises them. The spherical, membrane-surrounded entity that remains is called a **protoplast.**

Using methods essentially equivalent to those described for hybridoma production, protoplasts can be fused together generating hybrid cells that exhibit properties in common to both the contributing cell types. Examples of the application of fusion methods to microorganisms include efforts to: (1) improve the antibiotic yield from Streptomyces strains; (2) analyze the genetics of brewer's yeasts; and (3) develop hybrid strains of fungi from the Aspergillus family to enhance citric acid production.

The application of protoplast technology to plants is a relatively recent development, but one that promises to revolutionize the food, agriculture, and forestry industries, and to have considerable impacts on the energy, chemical, and pharmaceutical industries. Scientists are now able to regenerate full grown plants from single cells or protoplasts. So far, only a few species of plant have been successfully cultured in this way, including tobacco, the Douglas fir, and carrots. But rapid advances in this field should soon make available this technology for most plants of commercial interest. This capability will occasion several advantages:

- mass production of clones of identical plants, each having the improved qualities of the original parent;
- rapid growth of the plant in tissue culture to the seedling stage of development, thus shortening generation times;
- a ready-made in vitro system for conducting genetic engineering in plants.

A great variety of plants produce chemical compounds that are highly useful to man. These compounds include drugs (such as digitalis, vitamins, steroids, and anti-cancer agents), rubber, and petroleum substitutes. The advent of plant cell and protoplast tissue culture technology makes possible the large-scale fermentation of plant cells in much the same fashion that microorganisms are currently grown in bulk. Useful plant products would be excreted into the growth medium and readily isolated. Future processes of this type will obviate the necessity of devoting large tracts of arable land to cultivation, and production costs should plummet.

The cell fusion procedures described in this section, both for plant and for animal cells, depend greatly for their success on the techniques of in vitro cell culture. Scientists have known for some time how to explant cells from particular organisms or tissues and to keep them alive for limited durations under sterile conditions in an incubator. Various nutrient media have been formulated and growth conditions established for a wide variety of plant and animal cells. A serious drawback to the large-scale commercial use of cell culture technology is its high cost, but future widespread industrial application, which seems likely, will introduce economies of scale, and continuing refinements in the techniques should lower costs.

### 2.2.3 Other gene-altering techniques

Several other methods exist for establishing new genetic material in microbes and in cells of higher plants and animals. Some species of bacteria possess a natural ability to exchange DNA by way of a process called **conjugation.** Extrachromosomal DNA, or **plasmids,** is especially mobile and is transferred between bacterial strains with considerable ease in some cases. (The ability of bacteria to develop resistance to many types of antibiotics is due to genetic information located on plasmids. Since these plasmids move about so freely, a number of bacterial strains pathogenic to man have become relatively refractory to antibiotic treatment.) Plasmids encoding distinct functions and residing in different bacterial strains can be combined into a single bacterium. Such a "superbug" was created by Chakrabarty, who at that time was working at General Electric. Plasmids from several strains of the species Pseudomonas, each capable of degrading a particular constituent of crude petroleum, were combined into a single cell, enabling the strain that resulted to digest several components of crude oil. This modified bacterial strain became the subject of a controversial patent application, the litigation of which eventually reached the Supreme Court (see Section 3.1.3.3).

An alternative to conjugation, which involves bacterium-to-bacterium transfer of DNA, is the process of **transduction**, in which viruses serve as transmitters of genetic material. When a virus infects a cell, normal metabolic activities cease, and processes are undertaken to mass-produce new virus particles. Part of this process involves replicating virus DNA and packaging it into protein shells. Occasionally, small portions of host cell DNA are carried along into the virus shells. After production of sufficient numbers of mature viruses, the host cell bursts, releasing the virus particles to initiate another round of infection. Cells infected in this second round will receive, in addition to viral DNA, the portion of DNA derived from the original host. Scientists have learned how to manipulate these processes so that specific DNA sequences (genes) are transferred, thereby endowing the recipient cells with properties previously inherent only to the initial hosts. The utility of transduction as a means of producing genetic alterations is well established using bacteria and bacterial viruses (bacteriophages). Recently, this general procedure has found application among higher animals. Considerable experimental work is being devoted to performing transduction in primates (e.g., monkeys) using the virus SV 40 ("SV" stands for "simian virus"). The eventual success of these studies has profound implications for genetic engineering in humans, with the prospects of curing genetic diseases, such as sickle cell anemia.

Genetic engineering in plants promises to be greatly stimulated in the years ahead owing to the existence of a bacterium called Agrobacterium tumefaciens. This microbe infects plant cells, giving rise to a plant tumor, called a **crown gall.**

The agrobacterium perpetrates this deed by transmitting to the plant cell a piece of its own genetic material, called **T-DNA**, which is part of a plasmid, namely the **Ti-plasmid** (for "tumor-inducing" plasmid). Copies of the T-DNA are incorporated permanently into the plant genes, and the agrobacterium is no longer needed. This instance of naturally occurring recombinant DNA provides a potentially very powerful tool for introducing foreign genes into plants.

### 2.3 Immobilized bioprocesses

Several techniques have evolved in recent years that have managed, to some extent, to exploit cellular biological processes on an industrial scale. These methods generally consist of confining, or immobilizing, intact **cells** or cellular **enzymes** within an inert matrix, followed by passage of substrate materials through this **bioreactor**. Chemical reactions (i.e., bioconversions) then take place that transform the substrate into more useful or less toxic products.

Enzymes are proteins that catalyze the chemical reactions of living cells. Like most proteins, they are relatively unstable and tend to lose their activity when exposed to denaturing conditions, such as heat, extremes of pH and salt concentration, the presence of surface-active agents (detergents) or heavy metals, and so forth. Immobilization procedures generally serve to protect enzymes from denaturation, thereby lengthening their useful lifetimes. A large number of inert support materials have been tested for various applications, including natural and man-made polymers, such as cellulose, starch, polyacrylamide, chitin, polyethylene, glass, and collagen. Enzymes can be either linked securely to the surface of the polymer or entrapped within a porous microcapsule. In order to maximize the reactive surface area, the support matrix can be fashioned into tiny beads or hollow fibers or semi-permeable membranes prior to affixing the enzyme. In all cases, successful operation of the bioreactor depends on maintaining a securely fixed, active preparation that, nevertheless, permits free movement of the substrates and products.

Current and potential applications of this technology are vast and will affect many industrial sectors. A few examples include:

- **Chemical industry**
  - alkene oxide production from corresponding glycols
  - surfactant production from glycerides
  - hydroxylation of carboxylic acids
  - amino acid synthesis
- **Energy industry**
  - hydrogen production from water using chloroplast enzymes
  - desulfurization of crude oil
  - biomass conversions into methanol or ethanol
- **Medical industry**
  - production of urocanic acid, a sunscreening agent
  - inter-conversion of various penicillin derivatives
  - steroid derivitizations
  - clinical analysis of blood and urine constituents (e.g., urea and glucose) by electrobiochemical reactions
  - synthesis of the antibiotic Gramacidin
- **Pollution control industry**
  - conversion of lignocellulosic wastes into useful products, such as glucose
  - biodegradation of toxic substances, such as PCB, kepone, dioxin, DDT, phenols
  - concentration of toxic heavy metals in waste streams

—air disinfection (e.g., for hospitals) using enzymes that destroy viruses and bacteria

—conversions of whey (waste product from dairy industry) to useful food products

—rotating biological discs for waste water treatment

—fixed-bed bioreactors for on-stream waste management

- **Food and agriculture industry**

  —milk coagulation (the first step in cheese production) using the enzyme rennet

  —production of high-fructose syrups from starch and cellulose for use as a sugar substitute

  —conversion of amino acid isomers to convert the non-nutritious D isomer into the L form

  —clarification of fruit juices and wines

Future developments in this area are likely to include improved methods for immobilizing live cells, especially microbes and plant cells. The process of **microencapsulation** promises to find considerable application here. Each microcapsule can be thought of as a tiny living colony in which cells divide and perform metabolic functions within the confines of the bead. Meanwhile, substrates pass through the beads and are converted into products which flow out of the system uncontaminated by cellular material. Moreover, the biocatalytic system would be self-regenerating since the micro-colonies within each capsule are undergoing continuous **turnover**; that is, dead cells are always being replaced by live cells. This form of reactivation never occurs with immobilized **enzyme** systems since isolated enzymes are not capable of self-rejuvenation and, upon inactivation, must be replaced.

Another likely development in the area of immobilized bioprocesses is the increased use of enzymes isolated from **thermophilic** bacteria. These microbes are remarkably insensitive to high temperatures, even up to 80° or 90° Celsius (water boils at 100°C). Thermophiles can be recovered from hot springs or other similar environments. They owe their heat resistance to having enzymes that are extremely insensitive to heat denaturation. Thus, these enzymes are considerably more stable than comparable enzymes from **mesophilic** organisms and are ideal for immobilization processes.

## 2.4 Fermentation technology

Commercialization of processes reliant on recombinant DNA or other modern biotechnologies will frequently entail large-scale microbial fermentations. Industrial fermentations have been carried out with great efficiency for many years and have made available at low cost such products as antibiotics, flavoring and coloring agents, amino and organic acids, and vitamins. The expectation that new drus, such as interferon and human insulin, will soon be mass-produced depends to a large extent on the ability of the fermentation engineers to adapt the appropriate microorganisms for growth in quantities vastly greater than those encountered in the laboratory.

A standard aerobic fermentor consists of a closed, cylindrical vessel equipped with a stirrer and internal baffles to provide agitation, heat exchangers to drain off the considerable heat generated during fermentative growth of the microbial culture, an aerator, and one or more inlets for media sampling and harvesting and exhaust gas removal. A device for rapid steam sterilization of the vessel is essential, as are controls designed to monitor and adjust growth conditions, such as temperature, air flow, pressure, pH, and degree of foaming. Crucial to the overall design is that no foreign microorganisms gain access to the system; all components are sealed to prevent leakage and can be steam-sterilized between batches. The fermentor can be of any convenient volume, ranging up to about 100,000 gallons, in which the media alone would weigh more than 400 tons.

A fermentor designed by Eli Lilly for large-scale growth of recombinant DNA organisms is shown in Figure 2-3. Incorporating design features such as exhaust gas filtration and double agitator seals, this reactor exceeds the safety and containment specifications of typical fermentors and, as such, sets the standard for fermentors designed for use with recombinant DNA organisms.

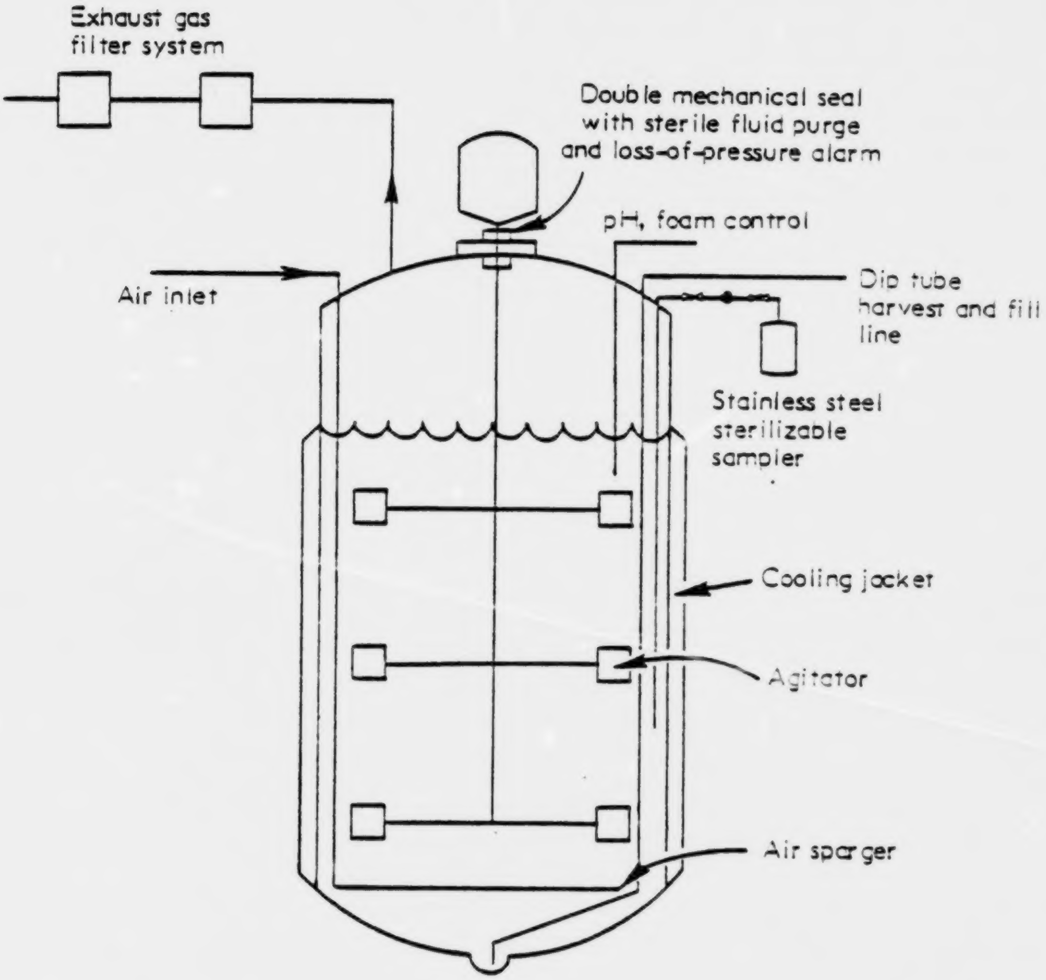
The fermentor described above carries out a **batch** fermentation; that is, the media and microorganisms are mixed, the microbes grow for a fixed period (usually one to seven days depending on the organism and the conditions), then the culture is harvested. After cleaning and sterilizing, the fermentor is ready for another batch. Following completion of the batch, the fermentation product must be isolated from the culture system. If the microbe excretes the desired product into the growth medium, as is preferable, then the culture broth must be processed following removal of the microbial population. On the other hand, products that accumulate within the microbe must be recovered by lysing the microorganisms after discarding or recycling the culture liquids.

Fermentation technology has advanced in at least two ways in recent years. It is now possible to conduct **continous** fermentations in which growth media are added slowly through the growing phase of the microbial culture. At the same time, small portions of the culture are continually removed from the fermentor for processing. The possibility of inadvertent contamination would seem to be greater for this continuous method, but efficiencies are greater since downtimes between batches are eliminated.

A second advance in this technology is called **solid phase** fermentation. In this process, nutrient media are trickled through a reactor consisting of a solid support matrix to which the microorganisms are steadfastly attached. The

Figure 2-3

Features of a contained fermentor


Exhaust gas filter system
Double mechanical seal with sterile fluid purge and loss-of-pressure alarm
pH, foam control
Air inlet
Dip tube harvest and fill line
Stainless steel sterilizable sampler
Cooling jacket
Agitator
Air sparger

Source: Eli Lilly and Co.

microbes continuously excrete the product of interest into the broth which eventually emerges at the bottom of the fermentor. The broth is then processed to isolate the fermentation product. Clearly, solid phase fermentation methods are not applicable to the mass-production of substances that accumulate inside the microorganisms.

## 2.5 Gene therapy

Perhaps the most exciting topic in the field of applied genetics, and likely the most controversial, is the probability that medical scientists will soon be able to perform genetic engineering in humans. Society in general views this prospect with a mixture of hope and skepticism, and assurances have been given that this capability is many years away. But recent scientific developments suggest that this future may be here quite soon.

Considerable attention was recently directed to the efforts of a team of UCLA scientists, headed by Martin J. Cline, who traveled to Israel and Italy to conduct experiments on human subjects. These experiments were deemed too preliminary to be performed in the United States. Those patients who were treated suffer from a genetic blood disease, called beta-thalassemia, in which production of one of the two protein components of hemoglobin is almost negligible. The therapy attempted to insert copies of normal genes for hemoglobin into cells of the bone marrow, where hemoglobin is synthesized. The experiment is given very little chance of success, but the mere fact that it was attempted, added to the fact that a similar experiment succeeded in laboratory animals, hints strongly that some primitive form of gene therapy in humans will shortly be possible.

In another recent development, scientists transplanted cell nuclei from early embryos of mice into fertilized eggs isolated from a different mouse strain.

After several days in tissue culture, these new embryos were inserted into the uterus of a third mouse. These embryos developed into normal infant mice that were related genetically to the mouse that orignally donated the cell nuclei. This outcome has been hailed as the first instance of **cloning** in mammals. That is, identical offspring were produced by taking cells, or cell nuclei, from a single individual and growing them up into complete, adult organisms. So far, it has not been possible to generate clones from **adult** donor cells--only cells derived from an early stage of development, such as the embryo, are suitable. But this stumbling block may soon be overcome. If so, and despite the claim by scientists that these experiments are designed only to study gene expression in mammals, then society will be faced with some very sticky ethical issues.

# SECTION 3

# INTERESTED PARTIES

## 3.1 Domestic activities

Immense excitement has been generated in recent years by the advent of recombinant DNA technology and the prospect that applied genetics will improve the quality of life in many ways. This interest arose from findings made in basic research labs at **universities**, which quickly burgeoned into a multi-million dollar **commercial** industry. Activities on both fronts are expanding continuously. Meanwhile, various **government** agencies have developed an interest in this area owing, in part, to concerns for public safety arising from overly fast commercialization of a technology whose safety has not been established absolutely. Thus, all three sectors--universities, private industry, and government--are deeply interested in the evolution of the applied genetics field. From a socioeconomic viewpoint, applied genetics will provide the opportunity to analyze and improve relationships between industries and universities on the one hand, and between industries and government on the other.

The remainder of this section of the report will detail activites in each of these three sectors in the United States, followed by a brief discussion of applied genetics as practiced overseas.

## 3.1 Universities

Most fundamental advances in both the science and engineering aspects of biotechnology have been made in university research labs. This fact will continue to hold true for some time to come, although considerable expertise is now being acquired by commercial firms engaged in applied genetics R&D.

A few of the many academic scientists who have contributed to the foundation of the applied genetics industry are listed in Table 3-1. This list, by no means exhaustive, includes many of those prominent academic scientists who have become affiliated with one or another genetic engineering firm. Several companies were founded through the efforts and energies of university researchers who, nevertheless, maintained faculty status at their academic institutions. This state of affairs has occasioned a certain degree of rivalry among university scientists who now view their research as potentially lucrative. As a result, the qualities of cooperation and intercommunication that once characterized academic research have been seriously compromised. This trend is likely to continue for the foreseeable future with accompanying improvements in industry-university relations at the expense of freedom of information flow within the scientific community. The situation could improve if private industries undertake programs to support basic academic research on an unrestricted, no-strings-attached basis. Commercial firms are being encouraged by Congress to do so via proposed tax credits and other investment incentives. Corporate backing of academic research has recently become especially desirable since federal sources of funds for basic biomedical research (i.e., NIH and NSF) have failed to keep pace with growing demand.

University faculties are organizing to offer their services as technical experts in applied genetics. Two examples are:

- **BioInformation Associates, Inc.** - A group of MIT professors from the biology, chemistry, and chemical engineering departments who provide wide-ranging consulting services for basic and applied research in genetic engineering.

- **Biotechnology Research Center** - Established at Lehigh University in Bethlehem, Pennsylvania, this joint effort of scientists and engineers provides education in biotechnology and conducts research in the areas of biomass conversions, microbial desulfurization of coal, and improved methods for waste treatment.

## Table 3-1

### A few academic scientists engaged in genetic engineering research; commercial affiliations (not inclusive)

| Name | University | Affiliation |
|---|---|---|
| Bert O'Malley | Baylor | |
| James Bonner | Cal Tech | |
| Leroy Hood | " " | |
| Gerald Fink | Cornell | advisor to Collaborative Genetics |
| Walter Gilbert | Harvard | co-founder of Biogen |
| Philip Leder | " | |
| Tom Maniatis | " | |
| Matthew Meselson | " | |
| Mark Ptashne | " | founder of Genetics Institute |
| Dan Nathans | Johns Hopkins | advisor to Monsanto |
| Hamilton Smith | " " | advisor to Cetus |
| David Baltimore | MIT | advisor to Collaborative Genetics |
| David Botstein | " | advisor to Collaborative Genetics |
| Arnold Demain | " | advisor to Cetus |
| Philip Sharp | " | co-founder of Biogen |
| Paul Berg | Stanford | |
| Stanley Cohen | " | advisor to Cetus |
| Ronald Davis | " | advisor to Collaborative Genetics |
| Roy Curtiss | Univ. of Alabama | |
| Martin Cline | UCLA | co-founder of AMgen |
| Winston Salser | " | co-founder of AMgen |
| John Baxter | UCSF | |
| Herbert Boyer | " | co-founder of Genentech |
| Anand Chakrabarty | Univ. of Illinois | |
| David Jackson | Univ. of Michigan | on leave to Genex |
| Stanley Falkow | Univ. of Washington | advisor to Cetus |
| Winston Brill | Univ. of Wisconsin | advisor to Cetus |
| Timothy Hall | " " " | advisor to Agrigenetics |
| Howard Temin | " " " | |
| Frank Ruddle | Yale | |

Harvard University recently developed, then rejected, a plan to establish its own genetic engineering company. This proposal evolved as a means to put the considerable talent of the Harvard faculty to the purpose of generating profits for the university, rather than to serve the interests of outside commercial firms (such as Biogen, co-founded by Harvard biologist, Walter Gilbert). The plan succeeded only in generating controversy. Faculty members argued that profit motives would add to the rivalry that already existed within the biology department and that traditional academic goals of education and research are imcompatible with a profit-making orientation. Eventually, the plan was abandoned, but Harvard biologist Mark Ptashne, who conceived the venture, proceeded to establish his own firm, called Genetics Institute, Inc., located in nearby Somerville, Massachusetts. Harvard owns approximately 10% of the equity of this new firm.

### 3.1.2 Commercial firms

The excitement generated by the field of biotechnology, particularly recombinant DNA and genetic engineering, has been felt most emphatically in the private sector of the U.S. economy. We have identified over 100 companies currently engaged in some aspect of modern applied genetics (see Table 3-2). More firms are becoming involved every month. It is estimated that capital investment in applied genetics R&D reached $500 million in 1980. In five more years, the value will be $5 billion, and in ten years, $25 billion. Many investors and business analysts anticipate that the decade of the 1980's will occasion a "biology boom" akin to the electronics explosion of the 1970's.

In general, capital investment in biotechnology has occurred along two different paths. Initially small, new companies specializing in genetic engineering were created by young scientists/businessmen who combined keen foresight with a propensity for financial risk-taking. These individuals anticipated the huge

## Table 3-2

## U.S. companies engaged in applied genetics R&D.

| Name | Location | Projects | Collaborators |
|---|---|---|---|
| I. **GENERAL** | | | |
| Cetus | Berkeley, CA | ethanol from biomass<br>alkene oxides/fructose<br>oil-related projects<br>interferon<br>single cell protein<br>peptide hormones | National Distillers<br>Chevron<br>Amoco<br>Shell Oil |
| Genentech | San Francisco, CA | interferon<br>insulin<br>growth hormone<br>alpha-1-thymosin<br>commercial scale-up | Hoffmann-LaRoche<br>Eli Lilly<br>A.B.Kabi (Sweden)<br>National Cancer Institute<br>Fluor<br>Lubrizol<br>Monsanto |
| Genex | Rockville, MD | interferon<br>chemical processes | Bristol-Myers<br>Koppers |
| Bethesda Research Laboratories (BRL) | Bethesda, MD | interferon<br>research enzymes<br>monoclonal antibodies<br>hepatitis vaccine<br>ethanol from biomass | New York Blood Center |
| Collaborative Genetics | Waltham, MA | interferon<br>industrial processes | Green Cross (Japan)<br>Dow Chemical<br>National Patent Development |
| EnzoBiochem | New York, NY | general biomedical | |
| Genetics Institute | Somerville, MA | general biomedical | Harvard University |
| AMgen (Applied Molecular Genetics) | Los Angeles, CA | general biomedical | Abbott Laboratories<br>TOSCO |
| Synergen | Boulder, CO | general biomedical | |

## Table 3-2

## U.S. companies engaged in applied genetics R&D.

| | Name | Location | Projects | Collaborators |
|---|---|---|---|---|
| I. | **GENERAL** (cont.) | | | |
| | DNA Science | New York, NY | provides venture capital | E.F. Hutton<br>Yeda R&D (Israel)<br>Battelle Columbus |
| | Alpha Therapeutic | Los Angeles, CA | vaccines | |
| | Armos | San Francisco, CA | instrumentation | (spin-off from Genentech) |
| | Atlantic Antibodies | Bar Harbor, ME | diagnostics | |
| | Becton Dickinson | Paramus, NJ | diagnostics | |
| | Bioassay Systems | Woburn, MA | interferon testing | |
| | Bio-Response | Wilton, CT | tissue culture | |
| | Biotech Research | Rockville, MD | diagnostics | |
| | Bio-Technical Resources | Manitowoc, WI | consulting | |
| | Brain Research | New York, NY | diagnostics<br>anti-tumor agents | (owns subsidiary in the Netherland Antilles to test drugs outside U.S. jurisdiction) |
| | Centocor | Philadelphia, PA | monoclonal antibodies | Wistar Institute |
| | Clonal Research | Newport Beach, CA | monoclonal antibodies | |
| | Cytogen | Edison, NJ | diagnostics | |
| | Damon Biotech | Needham Heights, MA | tissue microencapsulation<br>monoclonal antibodies | Harvard University<br>MIT |
| | Eastman Kodak | Rochester, NY | diagnostics<br>instrumentation | |
| | Electro-Nucleonics | Fairfield, NJ | diagnostics<br>vaccines | |

## Table 3-2

## U.S. companies engaged in applied genetics R&D.

| | Name | Location | Projects | Collaborators |
|---|---|---|---|---|
| I. | **GENERAL** (cont.) | | | |
| | Energetics | Palo Alto, CA | instrumentation | |
| | Flow General | McLean, VA | interferon<br>tissue culture | MIT Cell Culture Center |
| | Genetic Systems | San Francisco, CA | instrumentation | |
| | Hem Research | Rockville, MD | interferon<br>tissue culture | Applied Medical Devices, Inc. |
| | Hybritech | LaJolla, CA | monoclonal antibodies | |
| | Immunotech | Tampa, FL | diagnostics | |
| | IntelliGenetics | Stanford,CA | computer software for gene sequence analysis | Stanford University |
| | Interferon Sciences | New York, NY | interferon | National Patent Development |
| | M.A. Bioproducts | Walkersville, MD | diagnostics | Organon |
| | Molecular Genetics | Minneapolis, MN | vaccines<br>industrial processes | American Cyanamid |
| | Monoclonal Antibodies | Palo Alto, CA | monoclonal antibodies<br>diagnostics | |
| | Neo-Bionics | Albuquerque, NM | diagnostics | |
| | New England BioLabs | Beverly, MA | research enzymes | |
| | Organon | West Orange, NJ | diagnostics | Microbiological Associates (M.A. Bioproducts) |
| | University Patents | Norwalk, CT | general biomedical | |
| | Vega Laboratories | Tucson, AZ | instrumentation | |

## Table 3-2

## U.S. companies engaged in applied genetics R&D.

| Name | Location | Projects | Collaborators |
|---|---|---|---|
| **II. PHARMACEUTICAL** | | | |
| Abbott Labs | North Chicago, IL | urokinase<br>antibiotics<br>vitamins | AMgen |
| Baxter-Travenol | Deerfield, IL | diagnostics | |
| Bristol-Myers | New York, NY | interferon | Genex |
| Eli Lilly | Indianapolis, IN | insulin<br>growth hormone | Genentech<br>University of California |
| G.D. Searle | Skokie, IL | interferon | subsidiary in High Wycombe, U.K. |
| Hoffmann-LaRoche | Nutley, NJ | interferon | Genentech |
| Meloy Labs | Springfield, VA | interferon | (subsidiary of Revlon) |
| Merck | Rahway, NJ | interferon<br>antibiotics | |
| Miles Labs | Elkhart, IN | hormones<br>wastewater treatments | (subsidiary of Bayer, A.G.) |
| Ortho | Raritan, NJ | monoclonal antibodies | (subsidiary of Johnson & Johnson) |
| Pfizer | New York, NY | interferon<br>agricultural products | |
| Richardson-Merrel | Wilton, CT | general biomedical | (subsidiary of Dow Chemical) |
| Schering-Plough | Kenilworth, NJ | interferon | Biogen (Switzerland) |
| Smithkline | Philadelphia, PA | general biomedical | |
| Southern Medical & Pharmaceutical | Brandon, FL | interferon | Key Energy Enterprises, Tampa |
| Squibb | Princeton, NJ | general biomedical | |

## Table 3-2

## U.S. companies engaged in applied genetics R&D.

| Name | Location | Projects | Collaborators |
|---|---|---|---|
| II. **PHARMACEUTICAL** (cont.) | | | |
| Syntex | Palo Alto, CA | general biomedical | |
| Upjohn | Kalamazoo, MI | interferon-inducing drugs | |
| Warner-Lambert | Morris Plains, NJ | tumor diagnostics | Penn State University |
| Zoecon | Palo Alto, CA | steroids production | |
| III. **INDUSTRIAL CHEMICALS** | | | |
| Agri-Business Research | Scottsdale, AZ | petrochemicals from desert plants | Arizona State University |
| Allied | Morristown, NJ | industrial/agricultural chemicals | Biologicals (Canada) |
| American Cyanamid | Wayne, NJ | general biomedical | Molecular Genetics |
| Celanese | New York, NY | methane from petrochemical wastes | |
| Diamond Shamrock | Tucson, AZ | oils and rubber from desert plants | University of Arizona |
| Dow Chemical | Midland, MI | general biomedical<br>industrial processes | Collaborative Genetics |
| DuPont | Wilmington, DE | interferon<br>general biomedical<br>chemical feedstocks | New England Nuclear (acquired by DuPont)<br>Cal Tech |
| W.R. Grace | New York, NY | industrial chemicals | |
| Monsanto | St. Louis, MO | industrial chemicals<br>general biomedical | Biogen (Switzerland)<br>Genex<br>Genentech |

## Table 3-2

## U.S. companies engaged in applied genetics R&D.

| Name | Location | Projects | Collaborators |
|---|---|---|---|
| **III. INDUSTRIAL CHEMICALS** (cont.) | | | |
| Pennzoil | Houston, TX | petrochemicals from plants | |
| Revlon | New York, NY | drugs from plants | Meloy Labs |
| Rohm & Haas | Philadelphia, PA | agricultural chemicals | Advanced Genetic Systems |
| Stauffer Chemical | Westport, CT | industrial chemicals | |
| Union Carbide | New York, NY | industrial chemicals | Oak Ridge National Labs |
| **IV. ENERGY** | | | |
| Arthur D. Little | Cambridge, MA | biofuels | DOE |
| Ashland Oil | Ashland, KY | ethanol from cornstarch | Publicker Industries |
| Bio-Gas | Arvada, CO | methane from animal wastes | Los Alamos National Labs |
| Dynatech R&D | Cambridge, MA | fuels from algae | |
| Ecoenergetics | Vacaville, CA | biofuels | |
| Exxon Research | Florham Park, NJ | general energy-related | |
| National Distillers and Chemicals | New York, NY | general energy-related | Cetus |
| Shell Oil | Houston, TX | petrochemical substitutes | Cetus |
| Standard Oil of California (Chevron) | San Francisco, CA | alternative sources of liquid fuels | Cetus |
| Standard Oil of Indiana (Amoco) | Chicago, IL | enhanced oil recovery<br>general energy-related | Cetus |
| Lubrizol | Wickliffe, OH | petrochemical substitutes | Genentech |

## Table 3-2

## U.S. companies engaged in applied genetics R&D.

| Name | Location | Projects | Collaborators |
|---|---|---|---|
| **IV. ENERGY** (cont.) | | | |
| Native Plants | Salt Lake City, UT | biofuels | Plant Resources Institute, Utah |
| Solar Energy Research | Golden, CO | photobiological energy conversion | |
| Phillips Petroleum | Bartlesville, OK | alternative sources of liquid fuels | |
| Synthetic Fuels Corp. | Washington, DC | fuels from biomass | DOE<br>numerous contractors |
| UOP | Des Plaines, IL | microbial chemical catalysts | National Oil Co. of Spain |
| **V. POLLUTION CONTROL** | | | |
| ARCO Chemicals | Philadelphia, PA | biological wastewater treatments | |
| Battelle Columbus | Columbus, OH | chemical detoxification | |
| Biochemical Corp. of America | Birmingham, AL | biological wastewater treatments | division of Sybron Corp. |
| Cytox | New York, NY | chemical detoxification | |
| Ecolotrol | | biological wastewater treatments | |
| Envirotech | Salt Lake City, UT | mineral leaching | |
| General Electric | Schenectady, NY | oil degradation<br>ethanol from biomass | |
| Jacobs Engineering | Pasadena, CA | biological wastewater treatments | |

# Table 3-2

## U.S. companies engaged in applied genetics R&D.

| | Name | Location | Projects | Collaborators |
|---|---|---|---|---|
| **V.** | **POLLUTION CONTROL** (cont.) | | | |
| | Polybac | Allentown, PA | activated sludge microbes | subsidiary of Cytox Corp. |
| | SRI International | Menlo Park, CA | chemical detoxification | |
| | Sybron Biochemical | Birmingham, AL | biological wastewater treatments | |
| **VI.** | **AGRICULTURE/FOOD** | | | |
| | Agrigenetics | Madison, WI | nitrogen fixation<br>crop development | |
| | Archer-Daniels-Midland | Decatur, IL | agricultural research<br>ethanol from cornstarch | |
| | A.E. Staley | Decatur, IL | fructose syrup | |
| | Calgene | Davis, CA | agricultural research | |
| | Campbell Soup | Camden, NJ | food products | |
| | Frito-Lay | Dallas, TX | food products | |
| | Genetic Engineering | Denver, CO | animal husbandry | |
| | International Plant Research Institute | San Carlos, CA | agricultural research | |
| | Litton Bionetics | Rockville, MD | agrichemicals<br>medicinals | |
| | Ralston Purina | St. Louis, MO | food products | |

commercial potential of modern biological techniques and managed to attract venture capital to underwrite their business plans. The two pre-eminent examples of this venture capital apporach are:

- **Cetus Corp.,** founded in 1971 by UC Berkeley physicist Donald A. Glaser, biochemist Ronald E. Cape (who also earned an MBA degree from Harvard), and Peter J. Farley (a medical doctor with an MBA from Stanford). Even before the advent of recombinant DNA techniques, Cetus funded its operations through contracts with larger commercial firms, especially pharmaceutical houses. Current backers of Cetus include major oil companies, such as Amoco, Chevron, and Shell.
- **Genentech, Inc.,** founded in 1976 by Robert A. Swanson (who holds degrees in chemistry and business management from MIT) and UCSF biochemist Herbert W. Boyer. The firm was established expressly to commercialize on DNA technology and was initially underwritten by venture capital, chiefly from Kleiner & Perkins in California, Wilmington Securities in Delaware, and Lubrizol Enterprises in Ohio. Genentech currently operates with capital derived from specific contracts with large firms, such as Hoffmann-LaRoche and Eli Lilly, and with capital derived from a recent public sale of stock.

The contribution of small, innovative companies such as these to the emergence of the applied genetics industry has been summarized by Nelson M. Schneider, a drug-industry investment analyst for E.F. Hutton:

> All major new technologies have been promoted and fostered by small companies. The small guys have the opportunity only because the bigger guys ignore it. The big companies can't see the forest for the trees. They choose not to participate because of their own ingrown bureaucracies.

Thus, small companies such as Cetus and Genentech, and the dozen or more similar young firms that have sprung up recently, have the flexibility and the expertise to take advantage of scientific advances in the applied genetics field. But today's small companies are determined to grow. Says Peter Farley, president of Cetus:

> It's biology's turn now. We actually saw it coming, and we were determined right from the outset to become a major company. It's not a get-rich-quick scheme. We expect to be around fifty years from now as a major company.

It remains to be determined whether Cetus, as a major corporation, can continue to "see the forest for the trees."

The second principal business strategy for investing in biotechnology has been for large, technically oriented companies to undertake independent, in-house R&D programs. Virtually every major U.S. pharmaceutical firm has engaged in or made plans to initiate recombinant DNA research. The high level of interest among firms in this industry stems from the obvious applications of the new technology to the manufacturing of new or improved drugs. Some drug firms have undertaken collaborative research ventures with small genetic engineering firms directed towards the development of specific products. Examples include arrangements between Genentech and Hoffmann-LaRoche to make interferon, Genentech and Eli Lilly to make human insulin, and Genex and Bristol-Myers to make interferon.

Large corporations representing other industrial sectors are also investing heavily in applied genetics. DuPont, the world's largest chemical producer, has undertaken a sizeable commitment to R&D in the biosciences. Likewise, several major oil companies, such as Amoco and Phillips, have established in-house programs in biotechnology. These large, wealthy companies are hiring high-quality scientists and bioengineers for the purpose of developing biological solutions for problems such as alternative sources of energy and petrochemical feedstocks. Collaborative agreements with small firms exist here too, such as contracts between Cetus and several oil companies, including Amoco, Chevron, and Shell, to conduct R&D on energy-related projects.

While small genetic engineering firms will continue to conduct laboratory-scale R&D, commercial scale-up of biotechnological processes will require capital investment that only large firms can undertake. Thus, the relative importance of large companies, with respect to the growth of the applied genetics industry, will increase at the expense of the smaller firms. A trend can be anticipated paralleling that which occurred in the semiconductor industry during the 1970's; namely, larger firms will acquire through merger (or drive out of business) the many small, specialized genetic engineering companies that have emerged.

The recent efforts of Cetus and Genentech to raise large sums of money by offering shares of stock to the public reveal the difficulty that small companies face when undertaking capital-intensive projects. A short time ago, the corporate management of both companies expressed desires to avoid going public with their stock until the mid-1980's at the earliest. However, several factors served to alter their plans: (1) commitments to pursue costly in-house programs of commercial scale-up; (2) the failure to attract additional financing from large corporate backers (such as Chevron and Amoco in the case of Cetus); and (3) the absence to date of saleable products derived from R&D investments. Public ownership may compel these companies to lose some of their flexibility and farsightedness that provides them with the competitive edge over large, bureaucratic corporations.

### 3.1.3 Federal government

The involvement of the federal government in applied genetics stems from a concern, first expressed by research scientists in the mid-1970's, that the application of recombinant DNA techniques could produce new organisms that might escape from the laboratory and endanger the human population and the environment. Thus, the role of the government in biotechnology has so far been limited to considering the practice of recombinant DNA methods in academic and commercial settings.

### 3.1.3.1 The National Institutes of Health

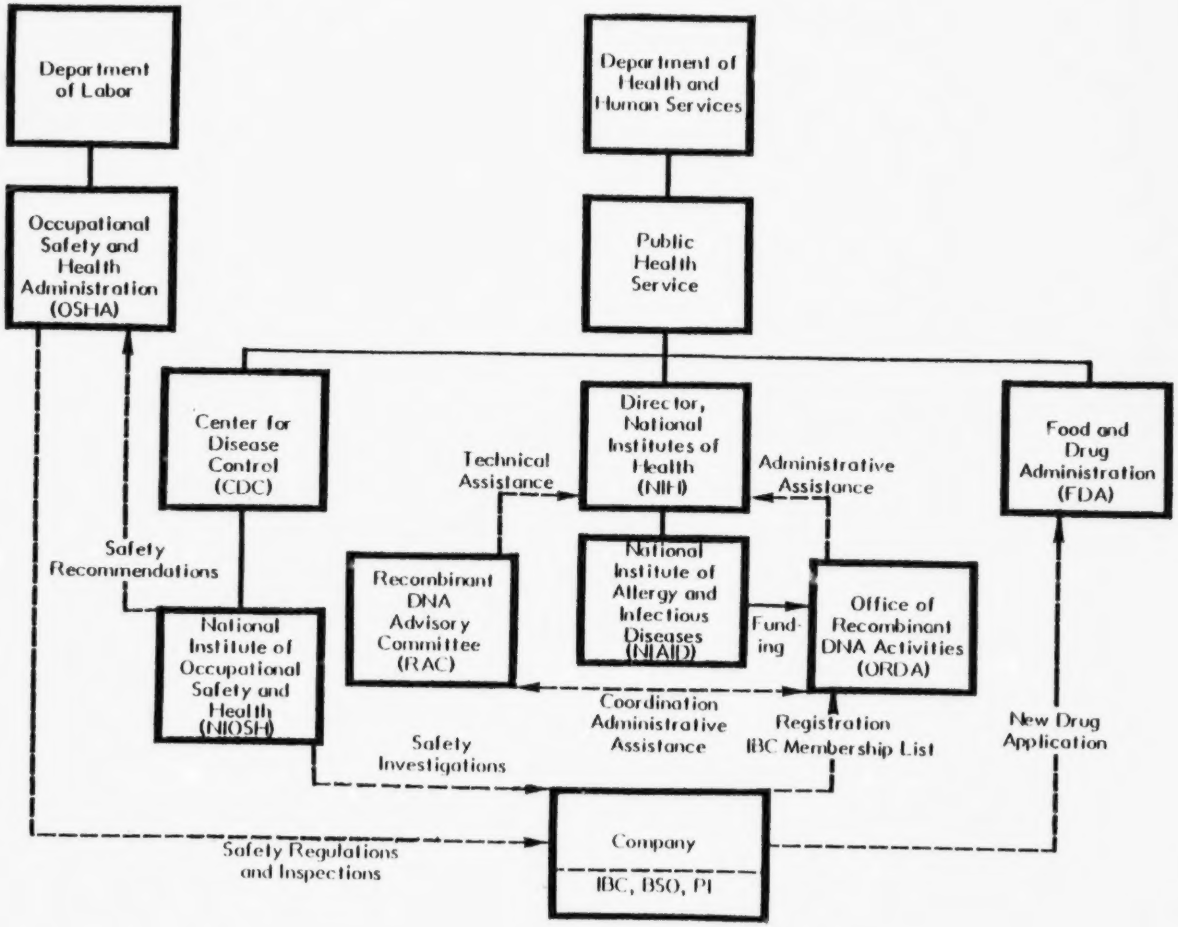
In the United States, the National Institutes of Health (NIH) control the administration of all federally supported recombinant DNA research and of all such activities carried out by commercial firms in voluntary compliance with NIH Guidelines for Research Involving Recombinant DNA Molecules. Other government agencies also involved in the potential regulation or control of commercial activities are the Food and Drug Administration (FDA), the Occupational Safety and Health Administration (OSHA), the National Institute for Occupational Safety and Health (NIOSH), and the Environmental Protection Agency (EPA). Figure 3-1 depicts the organizational relationships between the various government agencies and any company involved in recombinant DNA activities.

The purpose of the NIH guidelines for recombinant DNA research is to specify proper practices for constructing and handling recombinant DNA molecules and for handling organisms and viruses containing such molecules. Compliance with the guidelines is mandatory for all institutions engaging in such research and receiving federal support. The guidelines were first published in the Federal Register in the summer of 1976. Since then they have been amended considerably and now reflect a more confident and relaxed attitude about potential risks inherent in these activities. The most recent version of the guidelines appeared in the November 21, 1980, issue of the Federal Register.

The director of the NIH is responsible for the establishment, implementation, and final interpretation of the guidelines. Pursuant to the guidelines, the

Figure 3-1

Government agencies involved in recombinant DNA activities


Department of Labor
Occupational Safety and Health Administration (OSHA)
Department of Health and Human Services
Public Health Service
Center for Disease Control (CDC)
Director, National Institutes of Health (NIH)
Food and Drug Administration (FDA)
Technical Assistance
Administrative Assistance
Safety Recommendations
Recombinant DNA Advisory Committee (RAC)
National Institute of Allergy and Infectious Diseases (NIAID)
Fund-ing
Office of Recombinant DNA Activities (ORDA)
National Institute of Occupational Safety and Health (NIOSH)
Coordination Administrative Assistance
Registration IBC Membership List
New Drug Application
Safety Investigations
Company
IBC, BSO, PI
Safety Regulations and Inspections

Director has established the Recombinant DNA Advisory Committee (RAC) and the Office of Recombinant DNA Activities (ORDA) to provide technical and administrative assistance in the fulfillment of these responsibilities.

The RAC was established to provide technical and scientific assistance to the Director of NIH. Consequently, its membership must collectively reflect expertise in scientific fields relevant to recombinant DNA technology and biological safety. Additionally, at least 20 percent of its members must be knowledgeable about applicable law, standards of professional conduct and practice, the environment, public and occupational health, and related fields. The RAC meets four times a year and advises the NIH Director on changing the containment levels specified for various types of experiments covered under the guidelines, assigning containment levels to experiments not covered by the guidelines, and recommending new host-vector systems. The recombinant DNA field has expanded rapidly over the past few years, especially with the increasing involvement of private industry. Consequently, the RAC has been compelled to assess large-scale fermentation procedures, to examine confidential industrial data, and to review occupational safety and health standards.

However, the RAC has little expertise in industrial engineering. Furthermore, as an advisory committee to a non-regulatory agency, it has no authority to require compliance with its advice. The RAC has recently decided to limit its assessment of industrial facilities to an examination only of the **biological** characteristics of the operation (45 FR 77379). Consequently, private commercial firms will no longer be requested to submit to the NIH the details of their physical plants, medical surveillance programs, environmental monitoring schemes, or emergency plans.

The Office of Recombinant DNA Activities (ORDA) is responsible for providing maximum access to information on every aspect of the recombinant DNA field. As the focal point for all information on such activities, it provides technical and administrative advice to any institution, agency, or individual within or outside the NIH. In addition, it serves as executive secretary to the RAC, publishes the Recombinant DNA Technical Bulletin, and reviews and approves IBC membership lists (see below). ORDA's responsibilities also include the scheduling and announcing of RAC meetings and the publishing of revised guidelines in the Federal Register. ORDA also distributes information regarding policy decisions relevant to recombinant DNA research, announcements of training courses dealing with experimental and safety issues, up-dating of approved host-vector systems and other experimental protocols, and publishes a bibliography of newly released articles on recombinant DNA.

The NIH guidelines were amended in January 1980 to include a section dealing with voluntary compliance by private commercial firms engaging in recombinant DNA activities. This action was taken as a compromise to proposed mandatory controls put forward by the FDA. A scheme of voluntary compliance encourages private companies to follow the same administrative and technical procedures that are required of any federally supported institution. However, all items of information provided to the RAC or to ORDA by complying companies are protected as trade secrets, thus prohibiting subsequent disclosure under the Freedom of Information Act. In addition, companies that comply voluntarily are asked to register all projects involving recombinant DNA technology.

In April 1980, the NIH published a set of guidelines dealing with large-scale applications of recombinant DNA methodology. These guidelines detail the physical containment requirements for the production of recombinant DNA organisms in volumes exceeding ten liters.

Any company engaged in recombinant DNA activities and in voluntary compliance with the NIH guidelines must establish in Institutional Biosafety Committee (IBC). Each firm's IBC must include at least five members, with a minimum of two (but no fewer than 20 percent) having no affiliation with the company.

The IBC members collectively must have expertise in recombinant DNA research and be capable of assessing the safety of such experiments and the risks to workers and the community. The non-affiliated members are to represent the best interests of the surrounding community with respect to health and the protection of the environment. Officials of state or local public health or environmental protection agencies, members of other local government organizations or persons active in community medical, occupational health, or environmental affairs are all eligible to serve on an IBC.

The primary responsibility of the IBC is to review all recombinant DNA experiments conducted by the company to ensure compliance with the NIH guidelines. The IBC review must include an assessment of the containment levels utilized as well as an evaluation of the facilities, procedures, training, and expertise of the personnel conducting the experiments. The IBC must also adopt emergency plans covering accidental spills and contamination resulting from recombinant DNA research.

In addition to an IBC, any firm or institution engaging in recombinant DNA R&D involving high levels of physical containment (P3 or P4) must appoint a biological Safety Officer (BSO). The BSO is a member of the IBC and is responsible for conducting periodic inspections of lab facilities, reporting to the IBC any significant violations of the guidelines, developing emergency plans, and interacting with the principal investigator (PI) in areas of lab security, technical and safety procedures, and adherence to the guidelines.

### 3.1.3.2 Other Federal agencies

Recombinant DNA technology is rapidly moving out of the exclusive domain of university research laboratories and into industrial laboratories and large-scale production facilities. Concurrently, government agencies other than the NIH are becoming increasingly involved with issues concerning environmental monitoring, worker safety and health, and product quality.

The **Food and Drug Administration** has been involved from the very beginning with industrial scale-up of recombinant DNA technology. This interest stems from the fact that the first commercial products emerging from this new technology are likely to be intended for human use; namely, insulin, human growth hormone, and interferon.

New drugs intended for human use must be certified by the FDA through the approval of two company-submitted forms: (l) a notice of Claimed Investigation Exemption for a New Drug (IND); and (2) a New Drug Application (NDA). Together these forms supply the FDA with proprietary information on drug composition, results of human and animal testing, and manufacturing procedures. As of June 1980, the position of the FDA was that drugs produced by recombinant DNA technolgoy could **not** be marketed under existing INDs or NDAs as simply changes in manufacturing technique.

Submission of an IND informs the FDA that a company has tested a potential new drug and that it will be testing it further. Required by the form is a statement of the methods, facilities, and controls used for the manufacturing, processing, and packaging of the new drug to establish and maintain appropriate standards of identity, strength, quality, and purity.

The NDA is a request for approval to market the drug. Although intended primarily to provide information on the results of clinical testing, the NDA also contains detailed information on the manufacturing of the drug. It covers all the information that the sponsor knows about the drug and often consists of thousands of pages.

The **Occupational Safety and Health Administration** (OSHA) was established under the Occupational Safety and Health Act of 1970. OSHA is a regulatory agency within the Department of Labor that is charged with developing and promulgating standards, formulating and enforcing appropriate regulations to maintain safe and healthful conditions in the workplace. OSHA has announced that it will develop a recombinant DNA regulatory policy over the next two years. This could prove to be a difficult task since hazards associated with this technology have remained speculative. Two recent events will further contribute to the difficulty that OSHA will encounter in efforts to regulate recombinant DNA technology. In a recent Supreme Court decision, reduced standards for exposure to benzene were disallowed owing to a lack of evidence that the existing exposure levels were dangerously high. Similarly, there exists no firm evidence of risk resulting from contact with recombinant DNA organisms (at any level of exposure). Secondly, OSHA has no authority to preview the technical details that a company intends to use in the large-scale production of recombinant DNA organisms. Until now, OSHA has obtained this information from the RAC, but, as mentioned above, the RAC no longer intends to gather this information.

The **National Institute for Occupational Safety and Health** (NIOSH) was established by the Occupational Safety and Health Act of 1970. NIOSH is a component of the Center for Disease Control under the Public Health Service and is authorized to conduct research and recommend workplace standards to OSHA. NIOSH is interested in the following areas relevant to recombinant DNA:

- Process operations with attendant potential for worker exposure;
- Engineering controls, such as physical containment design, ventilation, exhaust gas filtration, waste product control, etc;
- Validation procedures pertaining to sterilization of equipment, physical containment, and process termination;
- Work practices, emergency and accident procedures, medical surveillance, environmental monitoring, and employee training and education.

### 3.1.3.3 Patent issues

The issue of patent protection for products and processes evolving from recombinant DNA R&D is both controversial and very important to commercial firms engaged in these activities. Two events have had a sizeable impact so far. One established the legal precedent that man-made microorganisms are not excluded from patent protection; the second extended patent coverage to the inventors of certain basic laboratory procedures.

On June 16, 1980, the U.S. Supreme Court ruled in a narrow 5-4 decision that the General Electric Corp. should not be denied patent protection on an "oil-eating" microorganism developed by Dr. A. M. Chakrabarty. The decision hinged on whether a microorganism is unpatentable subject matter simply because it is alive. The Court found that the principal criteria upon which an invention is deemed patentable (namely, that it be new, useful, and non-obvious) were in no way infringed by the fact that the invention is alive. The dissenting minority argued that those who originally framed the patent statutes never intended that patent protection be afforded to living things. The Court admitted to a lack of competence in evaluating the potential dangers or benefits of this new technology, and they further declared that any binding policy regarding patentability of living organisms must originate in Congress.

On December 2, 1980, Stanford University and the University of California were jointly awarded a patent dealing wth gene cloning techniques used in recombinant DNA experiments. The techniques, developed by Stanley Cohen at Stanford and Herb Boyer at UCSF, have become the basis for virtually all recombinant DNA experimentation to date. The two universities have declared that they will license the technology to any company that wishes to employ the techniques and they will collect royalties on its use. They have further stated that a condition for use of the technology will be adherence to the NIH guidelines. However, the patent applies only to the use of recombinant DNA technology within the borders of the United States and would not cover overseas operations by U.S.-based companies, or by foreign firms. It is highly unlikely that this patent could withstand a legal challenge if it is deemed to inhibit the commercial development of recombinant DNA technology. Moreover, litigants will argue that numerous refinements to the basic techniques have been made so that the original inventors are no longer entitled to patent protection.

### 3.2 Foreign activities

The overseas practice of applied genetics has proceeded in a fashion similar to its evolution in the United States. Much of the basic biological research that gave rise to this new industry occurred in foreign laboratories, particularly in Western Europe. As in the United States, there has emerged in several countries a variety of small new genetic engineering companies. Likewise, established corporations are engaging in applied genetics R&D. We are aware of over forty foreign companies, large and small, that have invested in biotechnology (see Table 3-3).

In contrast to U.S. activities, however, some foreign governments have supplied considerable financial backing to fledgling genetic engineering companies. For example, the **British** government, in concert with four London investment firms, has established Celltech. This nationally owned venture came into being only after extensive hand-wringing on the part of government planners, but Celltech can now hope to commercialize significant scientific achievements of British researchers, several of whom have already lost the opportunity to capitalize on their findings owing to a lack of public interest. (For example, the monoclonal antibody technique was discovered in England, but the scientists involved failed to patent the process within the necessary time limits.)

With regard to applied genetics in **France**, the government there has fostered a healthy relationship between universities and industries, thereby facilitating the transfer of basic biotechnology from academic labs into the commercial sector. The French government is committed to spend about $25 million over the next five years in support of biotechnology. Several French government science and research agencies have cooperated in support of a new business venture, called G3, which will concentrate on biomedical applications of genetic engineering.

In **Japan,** where government-industry cooperation is legendary, over a dozen established chemical and pharmaceutical firms are actively pursuing genetic engineering programs with government support. The Japanese are considered world leaders in certain areas of biotechnology, particularly fermentation techniques. They are far ahead of the rest of the world with regard to the quantity and diversity of products, such as antibiotics, vitamins, and food additives, that can be readily manufactured by fermentation procedures.

Most of the commercial development of genetic engineering in **Canada** has preceeded via private investment. A small new firm, BioLogicals in Toronto, recently signed a multi-million dollar agreement with Allied Chemical (a U.S. firm) to conduct applied research into the uses of genetic engineering for the production of industrial and agricultural chemicals. Connaught Laboratories, formerly associated with the University of Toronto and the site where the hormone insulin was first isolated in 1921, has been largely taken over by the Canadian government. The firm is now engaged in an ambitious revitalization program that includes large-scale investment in genetic engineering.

In **Israel,** biotechnology is being applied to meet national needs in the areas of agriculture, industrial chemicals, and waste management. Considerable effort is being expended to investigate various types of photosynthetic algae as potential sources of single cell protein and useful biochemicals. Genetically engineering salt tolerance into algae, thus allowing the microbes to thrive in brackish ponds, has received special attention.

For the most part, government regluation (or pseudo-regulation) of recombinant DNA activities in foreign countries has followed a path similar to that in the United States. Actual legislation dealing with this area exists only in the United Kingdom, where the GMAG (Genetic Manipulation Advisory Group) reviews experimental protocols much as does the RAC in this country. In Britian, however, emphasis has been placed solely on physical containment of recombinant DNA organisms, rather than on both physical and biological containment, as in the United States. Other Western European nations have generally followed the model set by GMAG.

The Japanese government has followed the U.S. lead in establishing voluntary guidelines for recombinant DNA research. The trend in Japan, as in all nations, has been to continually revise downward the restrictions imposed by the guidelines as information accumulates indicating biohazards inherent to recombinant DNA techniques are no greater than the risks associated with microbiological methods in general. Governments in all nations are fearful that unnecessary regulation of genetic engineering may adversely affect the commercial potential that this new technology offers.

# Table 3-3

## Foreign companies or government agencies engaged in applied genetics R&D

| Name | Projects | Collaborators |
|---|---|---|
| **CANADA** | | |
| Biologicals | industrial/agricultural chemicals | Allied (U.S.) |
| Connaught Labs | vaccines | (67% government-owned) |
| Inco | industrial chemicals | Biogen (Switzerland) |
| Levochem Industries | amino acids | (subsidiary of Commercial Organics, Canada) |
| National Research Council of Canada | industrial processes | Ontario Paper (Canada) |
| Sybron Biochemical | industrial waste treatment | Paper Research Institute of Canada |
| **UNITED KINGDOM** | | |
| Burroughs-Wellcome | interferon (tissue culture) | |
| Celltech | medical diagnostics | (100% government-owned) |
| Fospur | biological wastewater treatment | |
| HTL Specialties | industrial chemicals | Tate & Lyle (U.K.)<br>Hercules (U.K.) |
| ICI (Imperial Chemical Industries, Ltd.) | single cell protein | |
| Sera Laboratories | monoclonal antibodies | |

## Table 3-3

## Foreign companies or government agencies engaged in applied genetics R&D

| Name | Projects | Collaborators |
|---|---|---|
| **FRANCE** | | |
| Chimie Industrielle | industrial chemicals | |
| Elf-Aquitaine | energy from biomass<br>amino acids<br>nitrogen fixation | |
| Genetica | pharmaceuticals | (subsidiary of Rhone-Poulenc, France) |
| G3 (Groupement de Genie Genetique) | hepatitis vaccine<br>hormones | Institut Pasteur<br>National Institute for Health and Medical Research (INSERM)<br>National Center for Scientific Research (CNRS)<br>National Institute for Agricultural Research (INRA) |
| Sanofi | interferon | Institut Pasteur |
| Transgene | pharmaceuticals<br>bioenergy | Paribas (France) |
| **WEST GERMANY** | | |
| Boehringer-Mannheim | enzymes | |
| Hoechst | synthetic peptides<br>hormones | Massachusetts General Hospital (U.S.) |
| Schering | pharmaceuticals | |

# Table 3-3

## Foreign companies or government agencies engaged in applied genetics R&D

| Name | Projects | Collaborators |
|---|---|---|
| **SWITZERLAND** | | |
| Biogen | interferon<br>industrial chemicals<br>mining | Schering-Plough (U.S.)<br>Monsanto (U.S.)<br>Inco (Canada)<br>Grand Metropolitan Foods (U.K.) |
| Ciba-Geigy | pharmaceuticals<br>agricultural chemicals | |
| Hoffmann-LaRoche | interferon | Roche Institute (U.S.) |
| Sandoz | biological insecticides | University of California |
| **OTHER EUROPEAN** | | |
| Alfa-Laval (Sweden) | ethanol from biomass | |
| Kabi (Sweden) | human growth hormone | Genentech (U.S.) |
| Novo Industri (Denmark) | human insulin | |
| Gist-Brocades (Netherlands) | industrial chemicals<br>amino acids | |
| Nizo (Netherlands) | ethanol from biomass | Dutch Institute for Dairy Research |
| **ISRAEL** | | |
| Koor Food | single cell protein | Weizmann Institute (Israel) |
| Yeda R&D | interferon | Ares Co. (Switzerland)<br>DNA Science (U.S.) |

## Table 3-3

## Foreign companies or government agencies engaged in applied genetics R&D

| Name | Projects | Collaborators |
|---|---|---|
| **JAPAN** | | |
| Ajinomoto<br>Asahi Chemical<br>Kyowa Hakko Kogyo<br>Mitsubishi Chemicals<br>Sumitomo Chemicals<br>Dainichiseika Chemicals | hormones<br>vaccines<br>amino acids<br>flavoring agents<br>industrial chemicals<br>microbial air deodorants | |
| Hiki | wastewater treatment | |
| Japanese Fermentation Research Institute | bacterial concentration of heavy metals | |
| Japanese Science and Technology Agency | general microbial biotechnology | |
| Mitsubishi Kakoki Kaishha | wastewater treatment | Beppu University (Japan) |
| Ringen Biological Research | pharmaceuticals | |
| Shionogi | insulin | |
| Green Cross | interferon | Collaborative Genetics (U.S.) |
| Takeda Chemical | interferon | |
| Toray Industries | interferon | Japanese Foundation for Cancer Research |

# SECTION 4

## INDUSTRIAL APPLICATIONS, TRENDS, POTENTIAL HAZARDS

This section contains an industry-by-industry analysis of biotechnology. Each industrial sector will be examined for:

- **Current activities** in applied genetics. Some speculation may be required inasmuch as certain information is held as proprietary by private industries.
- **Future prospects** for the application of biotechnology within each industry.
- **Assessment of potential hazards** of applied genetics both as practiced currently within each industry and as possible future uses unfold.

The following commercial sectors will be examined: (1) pharmaceuticals, (2) industrial chemicals, (3) energy, (4) mining, and (5) pollution control.

### 4.1 Pharmaceutical industry

### 4.1.1 Current activities

The largest efforts to date towards commercial application of modern biological techniques have taken place in the pharmaceutical industry. The manufacture of new or improved drugs and vaccines surely will be the first commercial fall-out from recombinant DNA technology. Scientists recognized very early in the development of these techniques the immediate potential for mass-producing human biologicals, such as hormones and serum proteins, for eventual use as therapeutic agents. If available at all, such agents traditionally have been isolated from animal sources, a practice frequently leading to shortages in supply or to variation in quality from batch to batch. Moreover, biologics derived from animals generally differ slightly in structure from the human form of the analogous compound, thus providing a less than optimal human therapeutic.

The production by modern biotechnological methods of specific pharmaceuticals will now be considered.

**Interferon** is a protein synthesized by most cells of higher organisms in response to virus infections. Its production and secretion in miniscule amounts by infected cells serves to "interfere" with the spread of the infection to healthy cells. Thus, the administration of interferon as a drug promises to be a potent anti-viral therapy. In addition, interferon has been shown to act as an anti-tumor agent for certain types of cancer. Its potential as a cancer drug is now under thorough investigation at several clinical centers in the United States, notably the M.D. Anderson Hospital and Tumor Institute in Houston.

The severe shortage of purified human interferon has hampered adequate testing of its therapeutic value, but scientists have succeeded in applying recombinant DNA techniques to create bacterial interferon "factories" that promise to increase greatly the supply of the drug, while reducing enormously its current cost of several thousand dollars per dose. A predicted market of $3 billion per year has lured numerous commercial firms, both in the United States and overseas, to invest huge sums in interferon production and testing. Some companies are pursuing tissue culture methods, rather than recombinant DNA techniques, to obtain usable quantities of interferon. The therapeutic and commercial values of interferon will likely be revealed within the next year or two.

**Insulin**, a hormone made in the pancreas, aids in the metabolism of sugar. It is composed of two small polypeptides, the A and B chains, which are composed of 21 and 30 amino acids, respectively. In the pancreas, **proinsulin** is made as a precursor to insulin. Prior to secretion, the proinsulin is converted to insulin by the enzymatic removal from the middle of the molecule of a stretch of 35 amino acids called the C chain.

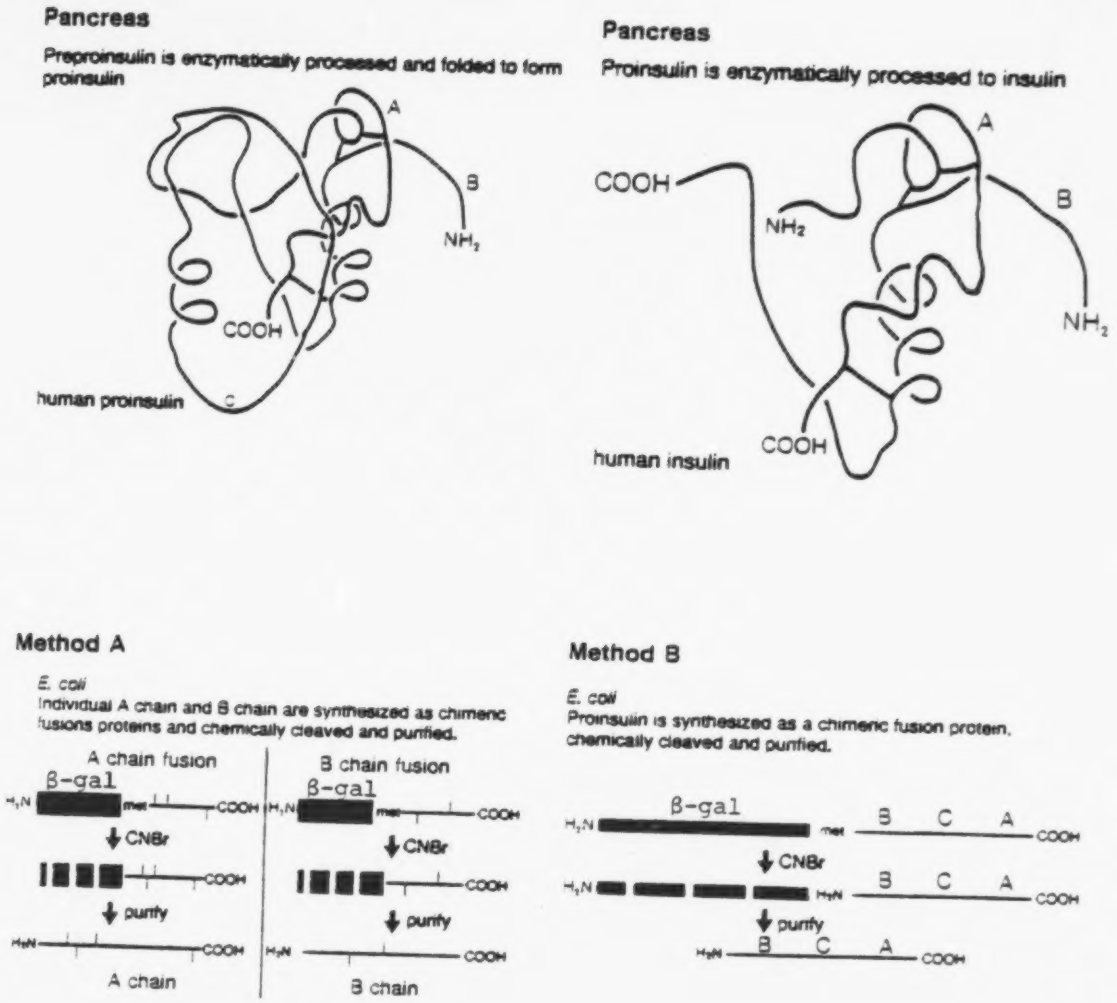
There are currently several strategies for producing insulin by recombinant DNA technology. Here we discuss two of them. In one, the genes for the A and B chains are chemically synthesized separately and inserted into separate plasmids as fusion proteins joined to the **lac operon** enzyme, beta-galactosidase. The gene to be cloned is a combination of the gene for the A or B chain and the gene for the enzyme, joined by the triplet codon for the amino acid methionine. The plasmid is then cloned in a bacterial host. Since neither the A nor B chain contains methionine, it can be efficiently removed from the fusion protein after the protein is extracted from the host. Removal is accomplished by treating the fusion protein with cyanogen bromide, which cleaves at the methionine juncture. The A and B chains are bound together as insulin by two disulfide bonds. After extraction from the enzyme proteins, they can be joined in the laboratory by using an air oxidation technique involving S-sulfonated derivatives and an excess of A chain. This methodology is 50 to 80 percent efficient in making the complete insulin molecule.

The second method utilizes only one organism to produce a fusion protein containing proinsulin. As in the first method, the extraction is made with cyanogen bromide. The isolated molecule is then treated with enzymes to remove the C chain, and the active insulin is recovered.

Figure 4-1 shows schematically the synthesis of proinsulin and insulin by recombinant DNA methodology.

Figure 4-1

Alternative methods for insulin production in E. coli


Pancreas
Preproinsulin is enzymatically processed and folded to form proinsulin
A
B
NH₂
COOH
human proinsulin
C
Pancreas
Proinsulin is enzymatically processed to insulin
A
COOH
NH₂
B
NH₂
COOH
human insulin
Method A
E. coli
Individual A chain and B chain are synthesized as chimeric fusions proteins and chemically cleaved and purified.
A chain fusion
β-gal
H₂N
met
COOH
CNBr
COOH
purify
H₂N
COOH
A chain
B chain fusion
β-gal
H₂N
met
COOH
CNBr
COOH
purify
H₂N
COOH
B chain
Method B
E. coli
Proinsulin is synthesized as a chimeric fusion protein, chemically cleaved and purified.
β-gal
H₂N
met
B C A
COOH
CNBr
H₂N
H₂N
B C A
COOH
purify
H₂N
B C A
COOH

Source: Ross, M.J. (1980)

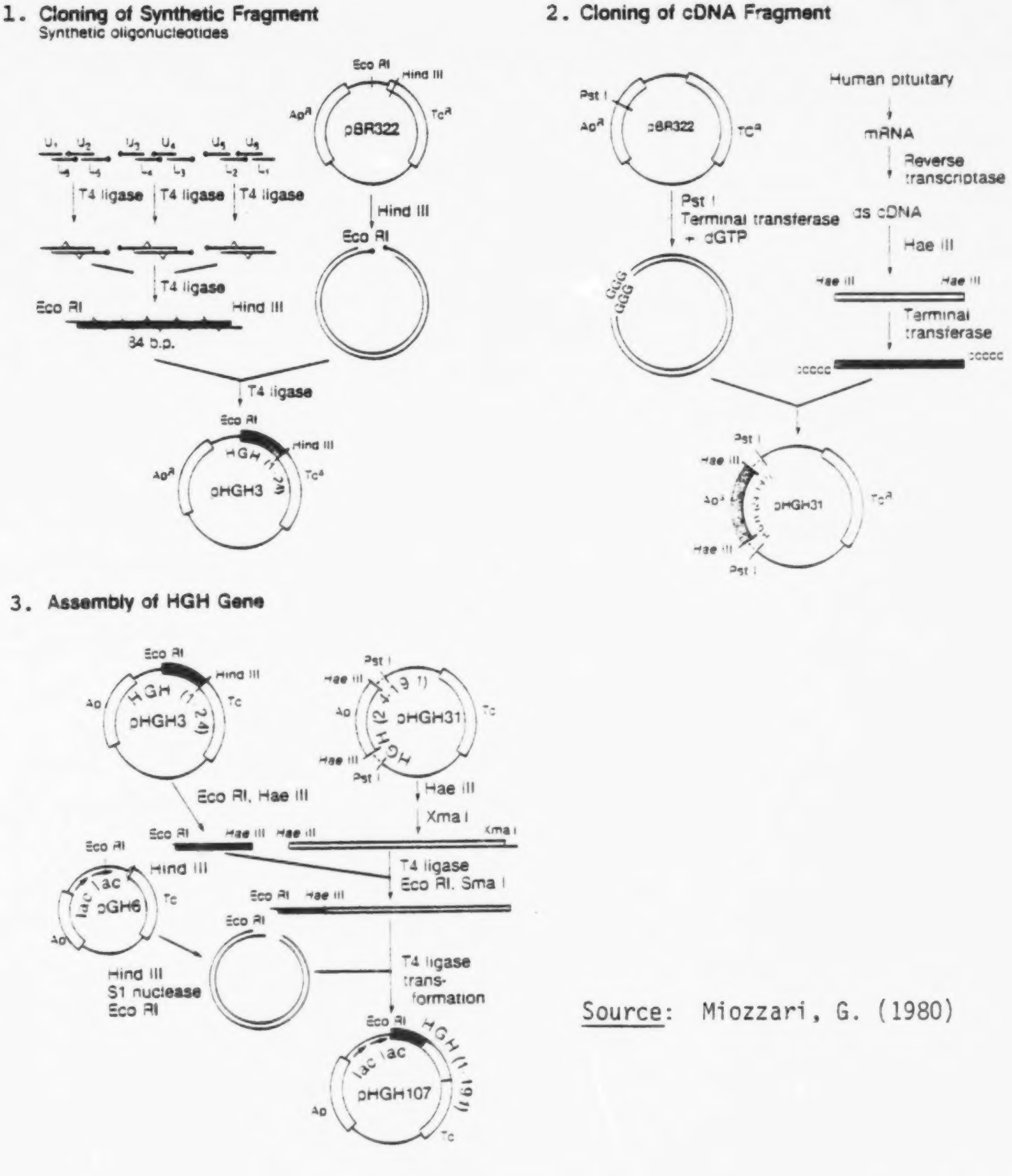
**Human growth hormone** (hGH) or somatotropin is produced in the pituitary gland and mediates growth and stature, particularly in children. The hormone traditionally has been extracted from the pituitaries of human cadavers (animal substitutes are not suitable) and is used in the treatment of dwarfism in children. Recombinant DNA technology offers the prospect of sharply increased supplies of the scarce hormone, leading to speculation that hGH will be useful in the treatment of a variety of disorders including ulcers, burns, bone fractures, and bone deterioration (osteoporosis, a common ailment of the elderly). Moreover, hGH may stimulate growth in a group of children (numbering close to a million in the United States) who are abnormally small despite having seemingly normal levels of circulating growth hormone. Clinical trials of "recombinant hGH" have just been initiated.

Human growth hormone has been sequenced in its entirety. The synthesis of an expression plasmid for bacterial hGH synthesis involved cloning a synthetic DNA fragment coding for the first 24 amino acids separately from a clone coding for the remaining 167 amino acids. The nonconjugable plasmid pBR322, which codes for resistance to the antibiotics ampicillin and tetracycline, was used as vector for both clonings. The combined hybrid gene for the entire 191 amino acids was fused to the gene for beta-galactosidase in the lac operon and then inserted into a new expression plasmid subsequently designated pHGH107. Figure 4-2 shows schematically the stages involved in constructing the final expression plasmid coding for the complete amino acid sequence of hGH.

The synthetic DNA segment coding for the 24 amino acids was constructed from 16 chemically synthesized lengths of DNA. These fragments were joined together using the enzyme T4 ligase. The resulting 84-basepair fragment was designed to have sticky ends by adding additional nucleotides at each end and then treating it with the restriction endonuclease enzymes Eco RI and Hind III .

Figure 4-2

Construction of a bacterial plasmid coding for the synthesis of human growth hormone


1. Cloning of Synthetic Fragment
Synthetic oligonucleotides
Eco RI
Hind III
Ap^R
pBR322
Tc^R
U1 U2 U3 U4 U5 U6
L6 L5 L4 L3 L2 L1
T4 ligase
T4 ligase
T4 ligase
Hind III
Eco RI
T4 ligase
Eco RI
Hind III
84 b.p.
T4 ligase
Eco RI
Hind III
Ap^R
HGH (1-24)
pHGH3
Tc^R
2. Cloning of cDNA Fragment
Pst I
Ap^R
pBR322
Tc^R
Human pituitary
mRNA
Reverse transcriptase
ds cDNA
Hae III
Pst I
Terminal transferase
+ dGTP
GGG
GGG
Hae III
Hae III
Terminal transferase
ccccc
ccccc
Pst I
Hae III
Ap^R
pHGH31
Tc^R
Hae III
Pst I
3. Assembly of HGH Gene
Eco RI
Hind III
Ap
HGH (1-24)
pHGH3
Tc
Pst I
Hae III
Ap
HGH (24-191)
pHGH31
Tc
Hae III
Pst I
Eco RI, Hae III
Hae III
Xma I
Eco RI
Hae III
Hae III
Xma I
Eco RI
Hind III
lac lac
pGH6
Tc
Ap
T4 ligase
Eco RI, Sma I
Eco RI
Hae III
Eco RI
Hind III
S1 nuclease
Eco RI
T4 ligase
trans-formation
Eco RI
HGH (1-191)
lac lac
pHGH107
Ap
Tc

Source: Miozzari, G. (1980)

*Eco* RI and *Hind* III were also used to open the vector, plasmid pBR322. The synthetic fragment was inserted into the plasmid and subsequently cloned. Then the plasmid with the correct DNA fragment was isolated from a cloned colony and designated pHGH3.

The 501-basepair cDNA fragment coding for the remaining 167 amino acids was prepared from pituitary mRNA, treated with the restriction endonuclease enzyme *Hae* III, and tailed with chemically synthesized segments of cytosine (C) nucleotides. The plasmid was treated with *Pst* I and joined to synthesized segments of guanine (G) residues. The vector and the fragment, rendered complementary under these conditions, were then joined together. Insertion and cloning followed.

In order to clone the complete gene, the two fragments were isolated from their plasmids and then joined together. The shorter, synthetic piece was cleaved from its plasmid with *Eco* RI and *Hind* III and then treated with *Hae* III to produce an *Eco* RI sticky end at one end of the fragment and a *Hae* III blunt end at the other. The larger cDNA fragment was then cleaved with *Hae* III and *Xma* I to produce a *Hae* III blunt end and an *Xma* I sticky end. The complete gene was made by joining the two *Hae* III blunt ends of the fragments with T4 ligase. Simultaneously, the *Xma* I end of the larger fragment was blunted with *Sma* I. This made that end suitable for insertion into a new plasmid (pGH6), as shown in the figure. This plasmid had been previously cloned with a copy of the lac operon. It was opened with *Eco* RI and *Hind* III and treated with S1 nuclease, thus leaving the plasmid with one *Eco* RI sticky end and one blunt end. The complete hGH gene was then fused to the lac operon, which permitted the expression of the hGH gene in the presence of lactose.

A number of other human peptides have been synthesized using recombinant DNA techniques. These include:

- **Somatostatin,** a short fourteen amino acid peptide hormone secreted by the hypothalamus, was the first human substance produced in bacteria. It may have therapeutic potential in the treatment of diabetes;
- **Thymosin,** a thymus hormone, regulates the development of a portion of the immune system. As a potential drug, it may influence the aging process and have application in cancer therapy;
- **Beta-endorphin** is a naturally occurring opiate that mimics the action of morphine. It has considerable therapeutic potential as a safe, non-narcotic pain-killer;
- **Urokinase,** a kidney enzyme, dissolves blood clots. It has potential as a drug to reduce the likelihood of heart attacks and strokes.

A second major pharmaceutical area in which recombinant DNA techniques are finding considerable application is in the development of new vaccines. Conventional vaccinations against viral diseases involve immunizing with inactivated virus particles, which stimulates the host's immune system to defend against a subsequent exposure to a live, active virus infection. The use of entire viruses as the immunizing agent, however, entails the risks that either the vaccine may elicit the disease (owing to incomplete inactivation), or that the vaccine will be ineffective as a result of denaturation of the virus during inactivation.

Medical scientists have acquired an understanding of the molecular basis of vaccination, so it has become possible to isolate the specific proteins from the outer surface of viruses that are responsible for stimulating an immune response. Injection of these proteins alone is sufficient to generate adequate immunity to the viruses, but the proteins are totally non-pathogenic; that is, they do not mediate an infection. Using recombinant DNA techniques, it has been possible to clone the viral DNA that directs the synthesis of these proteins and to gain expression of the genes in bacteria so that the proteins are manufactured. Such research has focused on efforts to generate vaccines to immunize against:

- **Hepatitis,** a serious liver disease that has reached epidemic proportions in some parts of the world;
- **Influenza,** the many forms of which have made reliable vaccines unobtainable using conventional techniques;
- **Foot-and-mouth disease,** a life-threatening disease among domesticated livestock.

In addition, vaccines are under development to combat certain pathogenic bacteria and the diseases that they cause, including:

- **Gonococcus,** which causes venereal disease;
- **Pathogenic E. coli,** which give rise to digestive ailments such as severe diarrhea, of life-threatening concern in infant children;
- **Oral bacteria,** which are responsible for tooth decay.

The discussion of applied genetics in the pharmaceutical industry has so far centered on the uses of recombinant DNA technology. A variety of other biotechnologies are finding application in this industry as well, including:

- Monoclonal antibodies for use as diagnostic agents for viral and parasitic diseases, such a hepatitis and malaria;
- New antibiotics generated by combining the synthetic capabilities of different antibiotic-producing strains;
- Bacterial production of chemical intermediates for use in drug synthesis, such as glutathione, a liver drug intermediate;
- The production of human serum proteins by tissue culture of cells derived from fusions between human embryonic cells and mouse liver tumor cells;
- The production of vitamin B12 by bacteria, which should prove more economic than its isolation from fungi, as currently practiced;

- Chemical modifications by microbes of drug intermediates, as in the synthesis of various antibiotics (streptomycin, penicillin, and gentamycin), and the transformation of steroids towards the manufacture of contraceptives;
- The use of higher plants and sea creatures for the production of steroids, antibiotics, atropine, digitalis, etc;
- A great variety of pharmacologically active agents can be isolated from naturally occurring microorganisms; a partial list is shown in Table 4-1.

### 4.1.2 Future prospects

It is the biomedical field where applied genetics will likely make the most dramatic, and most controversial, future impacts. The prospect of genetic engineering in humans raises deeply personal ethical questions that are not of concern to applications of biotechnology to other commercial sectors. As is the case with other new technologies, however, specific developments are difficult to predict; often the most significant applications are not even conceived of several years in advance. Nevertheless, certain trends are apparent that will direct the course of commercial activity in the biomedical area over the next few years at least.

- Interferon is not a single substance, but exists in multiple forms (numbering at least eight so far). The physiological role of each of these interferons has yet to be unraveled, but a better understanding of this biological system will lead to a wide variety of new drugs for treating specific viral diseases and some cancers. A note of pessimism: patients on long-term interferon therapy will probably develop resistance to the drug, much as chronic use of some antibiotics and anti-malarials has reduced the effectiveness of these drugs. Thus, continual development of new chemical forms of interferon will be needed.
- There will be a resurgence in the search for natural drug-like substances produced by plants and sea creatures. A

## Table 4-1

## Examples of pharmacologically active natural products isolated from microorganisms

| Activity | Product | Producing strain |
|---|---|---|
| anticoagulant | Phialocin | *Phialocephala repens* |
| antidepressant | 1,3-Diphenethylurea | *Streptomyces* sp. |
| anthelmentic | Avermectin | *Streptomyces avermitilus* |
| antilipidemic | Ascofuranone | *Ascochyta viciae* |
| antipernicious anemia | Vitamin $B_{12}$ | *Streptomyces griseus* |
| coronary vasodilator | Naematolin | *Naematoloma fasciculare* |
| detoxicant | Detoxin | *Streptomyces caespitosus* |
| DNA transformation inhibitor | Antraformin | *Streptomyces* sp. |
| esterogenic | Zearalenone | *Gibberella zeae* |
| food pigment | Monascin | *Monascus* sp. |
| herbicide | Herbicidin | *Streptomyces saganonensis* |
| hypotensive | Fusaric acid | *Fusarium* sp. |
| immune enhancer | N-acetylmuramyl tripeptide | *Bacillus cereus* |
| insecticide | Piericidin | *Streptomyces mobaraensis* |
| miticide | Tetranactin | *Streptomyces aureus* |
| plant hormone | Gibberellic acid | *Gibberella fujikuroi* |
| salivation inducer | Slaframine | *Rhizoctonia leguminicola* |
| serotonin antagonist | $HO_{2135}$ | *Streptomyces griseus* |

Source: Woodruff, H.B. (1980) Science, 208:1228.

variety of powerful drugs (e.g., digitalis, morphine, vincristine/vinblastine cancer drugs, and many steroids) were first isolated from plants. Modern drugs based on these compounds are now chemically synthesized. There exist numerous natural products that may serve as useful drugs but which occur in such limited quantities or which are so difficult to synthesize that commercial development is unlikely. Applied genetics will soon permit mass production of these substances by genetic manipulation of the organisms that produce them.

- Monoclonal antibodies ("hybridomas") have so far been produced only in mice. Mouse antibodies are inappropriate for use as human therapeutic agents, but recent developments have extended the hybridoma technique to permit the production of **human** antibodies. Such antibodies will have multiple drug uses: as antidotes for acute bacterial or viral infections; as agents for localizing and treating inaccessible tumors; as an adjunct to tissue transplantation to prolong graft survival; as safe contraceptive agents. Although the array of potential applications of hybridoma technology is considerably smaller than that of recombinant DNA methodology, drugs based on monoclonal antibodies will appear on the market in greater variety and with shorter delays than will the products of gene-splicing techniques.

- Recombinant DNA methods include the ability to transfer human genetic material into bacteria. This capability depends on certain bacterial **vectors**, usually plasmids or viruses, that carry the foreign DNA into the host microbe. Similarly, transfer of human DNA into other **human** cells or tissues is feasible through use of appropriate vectors that mediate the exchange. Such vectors, namely mammalian viruses, are under development; their availability will facilitate genetic engineering in humans. More serious technical questions stand in the way of eventual medical application, however. For example, which human genes should be transferred in order to treat which disease, and how are those genes isolated? What steps are required to establish those new genes in the recipient individual? Apart from technical obstacles, unresolved political and ethical issues pertaining to genetic experimentation in humans are certain to forestall widespread application of this technology for years to come.

### 4.1.3 Potential hazards

The range of potential health and environmental hazards posed by applied genetics will vary depending on the industrial setting. In the pharmaceutical and chemical industries, for example, processes involving genetically engineered microorganisms are likely to be contained within closed reactors or fermentors. Many applications of biotechnology in the mining and pollution control industries, on the other hand, foresee deliberate release of microbes into specific open environments. These two general modes of operation clearly impose different risks on (1) the health of the workers involved and of the surrounding community and on (2) the local ecology.

The application of biotechnology in the pharmaceutical industry gives rise to potential hazards at several levels of activity:

- The research laboratory, where scientists and technical personnel engage in the initial stages of development of new drugs or therapeutic regimens. A potential risk arising from the creation of new microbial strains via recombinant DNA techniques, for example, will be first experienced by laboratory personnel. The specific hazards involved are mitigated, however, by the high level of personnel training in general laboratory safety and by the relatively small quantities of material encountered in the laboratory setting.

- The production facility, where large-scale manufacturing, product isolation, and packaging processes are undertaken. The drug industry has amassed considerable experience in the safe operation of huge fermentation facilities. There remains the potential risk, however, of exposing the workplace (and to a lesser extent, the surrounding community) to aerosols containing viable microorganisms. Although their health is monitored quite closely, production workers are less able than are highly trained lab personnel to recognize the symptoms of microbial infection. Certainly, individuals residing in the surrounding community are generally unqualified to appreciate the risks posed by these activities.

- The end users, including medical personnel and patients, of drugs manufactured through applied genetics. Risks here are minimized by (1) enforcement of existing government regulations pertaining to the introduction of new drugs and biologicals and by (2) strict control of product quality by the manufacturer.

Microbiology laboratories have been examined since the turn of the century as sources of bacterial and viral infections. A recent survey (see R. M. Pike, 1976) summarizes nearly 4000 lab-associated infections dating back to the early 1950's. The most common bacterial and viral diseases reported among lab workers were brucellosis, typhoid, tularemia, and hepatitis. However, fewer than 20% of these infections could be associated with a known laboratory accident of any kind. (The lab practices most frequently giving rise to infections are mouth pipetting and the use of needles and syringes.) Although the incidence of such infections among lab workers is 5- to 10-fold higher than their frequency in the general population, the local community surrounding a microbiology lab appears to be at no greater risk than the population at-large. For example, despite 109 lab-associated infections at the Center for Disease Control during the period 1947-1973, no secondary cases were reported in family members or community contacts. In sum, these data suggest that, while workers in microbiology labs are exposed to increased health hazards, the risk to the surrounding community is minimal.

As mentioned previously, applied genetics, especially recombinant DNA technology, has received more commercial promotion in the pharmaceutical industry than in other commercial sectors. For this reason, assessments to date of the potential risks arising from this new technology have been made in the context of laboratory and industrial practices pertinent to the pharmaceutical sector.

A number of risk assessments have been conducted attempting to evaluate the safety of using E. coli K12 as a host bacterium for the manufacture of human proteins via recombinant DNA techniques. Three conferences dealing with this issue have been held: (1) at Falmouth, Massachusetts, in June 1977; (2) at Ascot, England, in January 1978; and (3) at Pasadena, California, in April 1980. The viewpoints expressed at these sessions are summarized as follows:

- The natural fragility of K12 would make it very difficult, if not impossible, for it to colonize the human gut, or to be communicated between individuals.

- The transfer into K12 of genes encoding the manufacture of virulent proteins (toxins) would not produce a fully pathogenic K12 strain, and the insertion into K12 of DNA from human viruses would present fewer risks than the same viruses existing freely in nature.

- The ingestion of a K12 strain that synthesizes and secretes a human hormone, such as insulin, would not contribute significantly to the hormone levels that occur naturally. Even assuming that more efficient hormone-producing strains are developed, current procedures require that the secreted protein be attached to extraneous material that is removed during commercial processing of the product, but which would prevent the formation of an active hormonal substance in the gut of an individual. Future technical advances, however, may obviate this safety feature.

- The bacterial synthesis of human proteins in the GI tract (or elsewhere in the body) would not likely trigger an auto-immune response to the human substance. That is, an individual infected with insulin-producing K12 would **not** produce antibodies to human insulin.

Thus, the great bulk of evidence indicates that E. coli K12 is eminently safe as a host bacterium for mediating the synthesis of human proteins. There currently exists no firm evidence conflicting with this conclusion. Although one can speculate as to the risks arising from the concurrence of a variety of unlikely events, the experience accumulated so far indicates strongly that the risks are minimal or non-existent.

Other microorganisms will soon be utilized as hosts for recombinant DNA procedures leading to the commercial production of drugs and biologicals. The two microbes most often discussed in this regard are a common soil bacterium, Bacillus subtilis, and brewer's yeast, Saccharomyces cerevisiae. As with E. coli, more is known of the genetics and molecular biology of these organisms than of any other microbes. Apprehensions regarding risks inherent in the use of these microbes have been far less than for E. coli K12. Neither B. subtilis nor S. cerevisiae cause serious infections in humans; only easily treated minor eye infections are attributed occasionally to B. subtilis.

Thus, these three microorganisms will likely underlie most commercial recombinant DNA activities within the pharmaceutical industry for the foreseeable future. Each of the three microbes has certain technical advantages and disadvantages that recommend its use on a commercial scale. The choice of which organism to use in a particular application will be made largely on economic grounds.

The NIH has recently approved the use of various species of Streptomyces as host organisms in recombinant DNA experiments (see 45 FR 50531). These microorganisms are especially important in the drug industry owing to their ability to manufacture the aminoglycoside class of antibiotics, including streptomycin, erythromycin, and tetracycline. The application of recombinant DNA technology to these microbial strains promises to generate improvements in product yield and, perhaps, to new and useful types of antibiotics.

NIOSH and NIH have examined the issue of worker safety in the pharmaceutical industry within the context of recombinant DNA activities. The NIH has proposed recommendations for large-scale fermentation of recombinant DNA organisms (analogous to the P1 to P4 designations for laboratory experimentation). Commercial firms are expected to comply voluntarily with these recommendations. So far, two U.S. firms, Eli Lilly and Genentech, have been granted NIH approval to proceed with scale-up operations.

In the spring of 1980, a NIOSH team conducted walk-through surveys of both these facilities. Eli Lilly operates a state-of-the-art commercial fermentation plant. All operations are closely monitored for leakage or contamination of biological material. Equipment is designed to minimize the formation of aerosols and to initiate sterilization procedures in the event of an accidental spill. Programs to ensure worker safety and health are in place, including medical surveillance, periodic safety inspections, monitoring employee work practices, and the provision of safety equipment and protective clothing.

Similar programs have yet to be instituted at Genentech, a firm that was founded in 1976 and has 100 years less experience than Lilly in large-scale fermentation operations. NIOSH, therefore, has recommended that Genentech plan immediately to implement similar safety and health protocols.

In summary, the pharmaceutical industry as a whole appears to be well equipped to deal with the various experimental and engineering safety issues that are posed by the advent of recombinant DNA technology. This industry historically has been involved in the "business of biology," and there exists a long tradition of safety associated with their operations. Moreover, a firmly established regulatory apparatus (largely housed in the FDA) already exists that closely monitors activities and screens new products originating from this industry. One must conclude that new products and processes stemming from various applications of genetic engineering in the pharmaceutical field will encounter the same careful scrutiny that has been devoted to conventional activities.

## 4.2 Chemical industry

### 4.2.1 Current activities

While not attracting public attention to the extent that interferon has, the chemical industry has been influenced by recent advances in biotechnology. Moreover, the industry may be on the verge of a technical revolution in which biological processes and renewable resources will rapidly replace the physical-chemical transformations of petroleum feedstocks upon which the industry is currently based. This section will attempt to outline some of the applications of biotechnology that are now in use and which serve as prototypes for the kinds of bioprocesses that may soon pervade this industry.

One chemical process utilizing biotechnology that has received some attention is under development by Cetus in conjunction with Chevron Oil. The process entails **oxidation of alkenes** to the corresponding alkene oxides. These end-products are utilized in enormous quantities for plastics manufacture; for instance, ethylene oxide and propylene oxide are the raw materials for the production of polyethylene and polypropylene, respectively. The Cetus/Chevron bioprocess consists of three enzyme-catalyzed steps, as follows:

I. $\textbf{glucose} + O_2 + H_2 \xrightarrow{1} \textbf{fructose} + H_2O_2$

II. $\textbf{propylene} + H_2O_2 + KBr \xrightarrow{2} \textbf{bromoisopropanol}$

III. $\textbf{bromoisopropanol} \xrightarrow{3} \textbf{propylene oxide} + KBr$

enzyme 1 = glucose oxidase

enzyme 2 = chloroperoxidase

enzyme 3 = halohydrin epoxidase

The process is now undergoing pilot plant scale-up. The plan calls for designing an immobilized enzyme bioreactor in which the three enzymes are stably linked to an inert matrix. A continuous flow process ensues in which starting materials are percolated through the reactor, and products (fructose and alkene oxide) are recovered at the reactor outlet. It remains to be seen if this process is economically competitive with conventional alkene oxidations. Moreover, since the alkene starting material will generally be obtained from petroleum feedstocks, the process fails to overcome the dependence on dwindling and evermore-costly oil supplies.

The general use of microbial **enzymes** in industrial processes (Table 4-2) is rapidly becoming a big business. One estimate places the 1985 market in enzyme technology at $500 million. The food industry historically has been the primary user of enzyme-based processes, and will continue in this role as demand increases for sweeteners derived from cornstarch and from other less conventional forms of biomass. But rising demand for gasohol will lead to further uses for enzymes in ethanol production. Three general classes of enzymes are finding increasing commercial use:

- **Amylases** break down polysaccharides, such as cellulose, and mediate biomass conversions;
- **Proteases** break down proteins and are used commonly in the food industry, for example as meat tenderizers;
- A miscellaneous group, which includes **oxidases** and **isomerases** capable of performing specific chemical transformations of substrates, may soon find considerable utility in the chemical industry.

These industrial processes utilize microbes as sources of biological catalysts (enzymes) that, in turn, convert organic starting materials into products. A large variety of microorganisms directly synthesize **simple organic chemicals** when grown on carbohydrate substrates (see Table 4-3). Since many of these

## Table 4-2

## Commercial uses of enzymes

| Enzyme | Uses |
|---|---|
| proteases: | |
| alcalase | detergent additive to remove protein stains |
| bromelain | meat tenderizer |
| papain | stabilize chill-proof beer; meat tenderizer |
| pepsin | digestive aid in precooked foods |
| trypsin, ficin, and streptodornase | wound debridement |
| rennin | cheesemaking |
| carbohydrases: | |
| amylase | digestive aid in precooked foods |
| amyloglucosidase | production of dextrose from starch |
| cellulase and hemicellulase | preparation of liquid coffee concentrates and conversion of cellulose to sugar |
| glucose isomerase | production of high-fructose syrups |
| invertase | prevention of sugar granulation |
| lactase | prevention of lactose crystals in ice cream |
| pectinase | clarification of wine and fruit juices |
| catalase | peroxide removal in cheesemaking |
| lipase | flavor production in cheese |
| lipoxygenase | bread whitening |

## Table 4-3

### Organic compounds obtainable by microbial fermentation

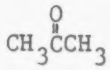
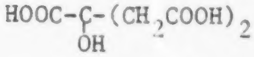
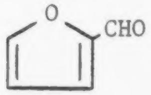
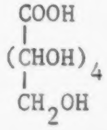
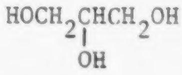
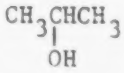
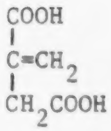
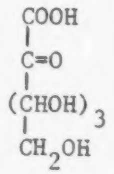
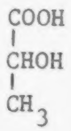
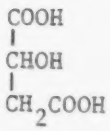
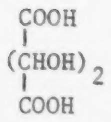
| Compound | Structure |
|---|---|
| acetone |  $CH_3\overset{\overset{O}{\Vert}}{C}CH_3$ |
| acetic acid | $CH_3COOH$ |
| acrylic acid | $CH_2{=}CHCOOH$ |
| butanol | $CH_3CH_2CH_2CH_2OH$ |
| citric acid |  $HOOC-\underset{OH}{\underset{\vert}{C}}-(CH_2COOH)_2$ |
| ethanol | $CH_3CH_2OH$ |
| ethylene glycol | $HOCH_2CH_2OH$ |
| furfural |  |
| gluconic acid |  $\begin{array}{l}COOH\\ \vert\\ (CHOH)_4\\ \vert\\ CH_2OH\end{array}$ |
| glycerol |  $HOCH_2\underset{OH}{\underset{\vert}{C}}HCH_2OH$ |
| isopropanol |  $CH_3\underset{OH}{\underset{\vert}{C}}HCH_3$ |
| itaconic acid |  $\begin{array}{l}COOH\\ \vert\\ C{=}CH_2\\ \vert\\ CH_2COOH\end{array}$ |

Table 4-3 (cont.)

| Compound | Structure |
|---|---|
| keto-gluconic acid |  $\begin{array}{l}\mathrm{COOH}\\ \mid\\ \mathrm{C{=}O}\\ \mid\\ \mathrm{(CHOH)_3}\\ \mid\\ \mathrm{CH_2OH}\end{array}$ |
| lactic acid |  $\begin{array}{l}\mathrm{COOH}\\ \mid\\ \mathrm{CHOH}\\ \mid\\ \mathrm{CH_3}\end{array}$ |
| malic acid |  $\begin{array}{l}\mathrm{COOH}\\ \mid\\ \mathrm{CHOH}\\ \mid\\ \mathrm{CH_2COOH}\end{array}$ |
| methanol | $\mathrm{CH_3OH}$ |
| propionic acid | $\mathrm{CH_3CH_2COOH}$ |
| tartaric acid |  $\begin{array}{l}\mathrm{COOH}\\ \mid\\ \mathrm{(CHOH)_2}\\ \mid\\ \mathrm{COOH}\end{array}$ |

compounds are toxic at relatively low concentrations, considerable research effort is being expended to generate microorganisms that tolerate higher doses of these organics. Also, modern fermentation technologies, such as continuous flow and solid state processes, will be useful here since metabolic products never accumulate to poisonous levels. In addition, the use of unconventional substrates for microbial fermentation, such as cellulose and lignin wastes, is rapidly becoming feasible. These technical advances may soon make economic bioproduction of these and many other organic compounds possible.

A number of microbial species growing on carbohydrate are able to synthesize **surfactants** or detergents. These compounds are typically long-chain fatty acids; current commercial production of surfactants requires petroleum feedstocks. The British sugar producer, Tate & Lyle, is now engaged in pilot-scale development of this process. Similar microorganisms are exploited for the production of polysaccharides for use in the food industry and the production of chemical flocculants, or precipitating agents, for use in sewage disposal.

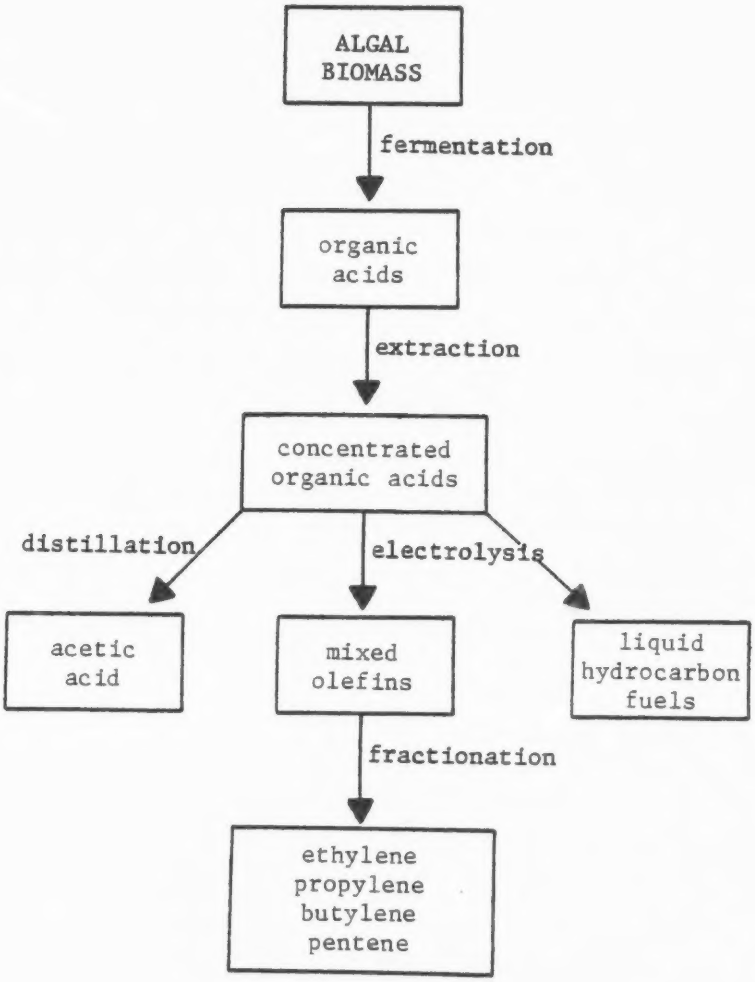
Photosynthetic **algae** offer the prospect of direct conversion of sunlight into useful organic chemicals. A considerable variety of end-products may be obtained from marine algae as shown in Figure 4-3.

Whereas certain microorganisms can synthesize various simple organic compounds, some **higher plants** have acquired the ability to manufacture rather complex molecules. As shown in Table 4-4, these substances include rubber and petroleum substitutes, insecticides, steroids, and other drug precursors.

The potential applications of genetic engineering in the chemical industry lie largely in the area of organics production. Many organic chemical feedstocks can be produced utilizing fermentation technology (see Table 4-3). In principle, the efficiency of microbes in any fermentative process can be improved by using

Figure 4-3

The extraction of useful chemicals from algae



ALGAL BIOMASS
fermentation
organic acids
extraction
concentrated organic acids
distillation
electrolysis
acetic acid
mixed olefins
liquid hydrocarbon fuels
fractionation
ethylene
propylene
butylene
pentene

Source: Sanderson, J.E., etal. (1979)

## Table 4-4

### Examples of useful chemicals derived from plants

| Plant | Substances | Uses |
|---|---|---|
| gopher plant (*Euphorbia*) | latex<br>sterols | rubber, petroleum substitutes, drugs |
| jojoba (*Simmondsia*) | long-chain esters | surfactants, emulsifiers, waxes, lubricants, preservatives, cosmetics |
| buffalo gourd (*Cucurbita*) | starch<br>linoleic acid | sweeteners<br>edible oils |
| guayule (*Parthenium*) | latex | rubber |
| scorpion flower (*Phacelia*) | latex<br>chromenes | rubber<br>insecticides |
| milkweed (*Asclepias*) | latex<br>silk-like fiber | rubber, chemical feedstocks, textiles |
| juniper (*Pinaceae*) | terpenoids<br>cadinene | antimicrobials<br>insecticides |
| *Varthemia candicans* | sesquiterpene lactones | antimicrobials |
| *Jotropha* | vegetable oils | surfactants |
| meadowfoam (*Limnanthes*) | fatty acids | surfactants |
| money plant (*Lunaria*) | fatty acids | surfactants<br>lubricants |
| bladderpod (*Lesquerella*) | hydroxy fatty acids | lubricants<br>ointments |
| thistle (*Chamaepeuce*) | hydroxy fatty acids | lubricants |
| kinkaoil ironweed (*Vernonia*) | epoxy fatty acids | plastics<br>coatings |
| hartleaf Christmasbush (*Alchornea*) | epoxy fatty acids | coatings |

recombinant DNA techniques or other biotechnologies. The extent to which biological processes will supplant chemical processes in the chemical industry will surely be a function of economics; the future cost of petroleum will be particularly influential. Though the entire chemical industry uses only 7 percent of the petroleum supply in the United States, this industry is heavily dependent upon this resource.

Fermentation technology is not new to the chemical industry. Prior to World War II (before the introduction of cheap oil), scores of chemicals were manufactured by fermentation processes. For example, only 36 percent of total ethanol production during the mid-1940's was based on petroleum sources; the remainder was made biologically. However, ten years later, almost 60 percent of the ethanol production was derived from oil. Fumaric acid was also manufactured on a commercial scale by fermentation. Production by this route ceased when a more economical synthesis from benzene was developed. In general, once a chemical process using petroleum was developed, it quickly replaced the existing fermentation process.

In spite of this history, a few chemicals are now produced by fermentation, notably citric acid, lactic acid, and various amino acids. These processes have all been improved over the years using applied genetics (e.g., microbial mutagenesis), but recombinant DNA technology has yet to have an impact in this area.

**Citric acid** is the most important acidulant in the food industry, representing 55-65 percent of the acidulant market. This acid also has pharmaceutical and chemical processing applications. Citric acid is produced commercially using the fungal organism, Aspergillus niger. The efficiency of this mold has been dramatically improved using mutagenic techniques. A four-fold increase in product yield has been obtained.

The bacterium Lactobacillus is used in the commercial production of **lactic acid.** Large quantities of this product are obtained using such raw materials as sucrose, glucose, and lactose (from cheese whey). Most of the problems in the manufacture of lactic acid exist in product recovery, not in the fermentative process itself. Thus far, biotechnology has been applied very little to improve this industrial process.

World production of **amino acids** is currently dominated by Japan; there is very little domestic U.S. production. The bulk of amino acids production is destined for research applications and to nutritional or biomedical preparations. Three amino acids are particularly useful: glutamic acid for the production of monosodium glutamate (MSG), a flavor enhancer; lysine and methionine as animal feed additives.

Glutamic acid production provided the first instance in which biotechnological methods were applied to enhancing amino acid production. The method involves the manipulation of microbial growth conditions and isolating mutant strains. Glutamate is produced in the presence of ammonia by a species of Corynebacterium. Growth of this particular species also requires the addition of biotin to the growth medium. In the presence of low concentrations of biotin, bacterial cell membranes become leaky to small molecules, thereby permitting glutamate to diffuse out of the cell. But at high biotin levels, the membranes are normal and prevent glutamate secretion. Furthermore, the biosynthesis of glutamate is reduced in the presence of high biotin levels through a feedback inhibition mechanism.

Lysine is produced both by chemical and fermentation processes. This represents one example where the chemical production method has not totally replaced the biological procedure. Due primarily to the lower direct operating costs incurred by fermentation procedures, about 80 percent of the lysine production world-wide in 1980 was via microbial means. The United States imported about 7,000 metric tons of lysine in 1979.

Recent announcements made by Bethesda Research Laboratories (BRL) indicate that recombinant DNA technology has been used to isolate some of the genes required in the synthesis of the amino acid proline. BRL is currently seekings ways to exploit this discovery on an industrial scale.

Table 4-5 lists those amino acids that are produced microbiologically and the bacterial species used in their manufacture.

### 4.2.2 Future prospects

The ease with which applied genetics has been integrated into the pharmaceutical sector is a result of that industry's predisposition towards the biological sciences. On the other had, the chemical industry depends largely on the technical disciplines of physical and organic chemistry and chemical engineering for its commercial foundation. Recent decades have seen remarkable advances in the mass production of industrial chemicals that have benefited society in numerous ways. Agricultural chemicals have improved food production, synthetic fibers have revolutionized the clothing industry, plastics influence our lives in countless ways, and so forth. But traditionally the chemical industry has not involved itself with biological processes. Only within the past few years have chemical firms, such as Allied, Dow, DuPont and Monsanto, undertaken programs to examine biotechnology as a way of doing business in the future.

A variety of issues relevant to the future of biotechnology in the chemical industry can be adduced.

## Table 4-5

### Fermentative production of amino acids from glucose

| Amino acid | Yield (gm/1) | Microorganism |
|---|---|---|
| DL-Alanine | 40 | *Corynebacterium gelatinosum* |
| L-Arginine | 29 | *Brevibacterium flavum* |
| L-Citrulline | 30 | *Brevibacterium flavum* |
| L-Histidine | 10 | *Brevibacterium flavum* |
| L-Homoserine | 15 | *Corynebacterium glutamicum* |
| L-Isoleucine | 15 | *Brevibacterium flavum* |
| L-Leucine | 28 | *Brevibacterium lactofermentum* |
| L-Lysine | 32 | *Brevibacterium flavum* |
|  | 44 | *Corynebacterium glutamicum* |
| L-Ornithine | 26 | *Corynebacterium glutamicum* |
| L-Phenylalanine | 2 | *Brevibacterium flavum* |
|  | 6 | *Bacillus subtilis* |
| L-Proline | 29 | *Brevibacterium flavum* |
| L-Threonine | 18 | *Brevibacterium flavum* |
| L-Tryptophan | 2 | *Brevibacterium flavum* |
| L-Valine | 23 | *Brevibacterium lactofermentum* |

- It is unlikely that biological processes will be applied in the near future to the large-scale manufacture of most commodity chemicals; i.e., bulk chemicals whose production capacity is measured in the millions of pounds annually. Although many of these products are derived from ever-more-costly petroleum feedstocks, bioprocesses will be unable to compete economically with traditional synthetic routes for 10-20 years or more. There exist obvious exceptions to this general conclusion, such as ethanol and some short-chain organic acids (see Table 4-3), but even these substances will be more cheaply produced by conventional methods for some time to come.

- A significant role for applied genetics in the chemical industry will be in the manufacture of high-priced specialty chemicals or in synthesizing new chemicals that have no practical alternative route. Enzymes will be employed as highly specific catalysts for performing discrete chemical steps in a synthetic route. Microorganisms that express the desired enzyme activity may be used directly. Microbes will be sought that carry out chemical transformations otherwise requiring large inputs of energy, such as hydrogenations, amidations, etc.

- The economics favoring the use of bioprocesses in the chemical industry will depend substantially on process design and engineering characteristics, rather than on the biotechnology involved. This is true for the chemical industry to a much greater extent than for the pharmaceutical industry. Thus, practical applications of biotechnology in this industrial sector will appear slowly and only following extensive analysis of the relevant biochemical engineering factors.

### 4.2.3 Potential hazards

The near-term role of applied genetics in the chemical industry predicts that bioprocesses will be developed that perform chemical transformations on specific feedstocks to manufacture specialty products. Consequently, the industry will be compelled to engage in large-scale microbial fermentations in order to obtain the necessary reagents (either the organisms themselves or the enzymes they synthesize) to perform these chemical reactions. Such fermentations, and subsequent product isolation procedures, will proceed in a manner entirely analogous to similar operations in the pharmaceutical industry. There exist differences, however, that may be of concern from an environmental or safety and health standpoint:

- The species of microorganisms likely to be utilized in the chemical industry differ from those in the drug industry. For example, various species of Pseudomonas, Acinetobacter, and Flavobacteria may find application in mediating chemical processes because these organisms naturally possess enzyme systems capable of catalyzing chemical reactions involving organic substrates (such as petroleum products) that are of interest to the chemical industry. Many of these microbes are opportunistic pathogens in man; that is, they infest skin lesions or cause severe infections in individuals who are already weakened by a pre-existing ailment.

- The chemical industry is unaccustomed to the application of biological processes as a business enterprise. Commercial-scale fermentations are alien to this industry. Chemical firms interested in adopting one or another bioprocesses may choose to purchase the technology, or to obtain the service through outside contract, rather than develop in-house capabilities.

- The chemical industry has a poorer record than the pharmaceutical sector in areas related to worker safety and environmental protection. This discrepancy may reflect the grossly different commercial operations performed by these two industries rather than neglect. Nevertheless, one might be apprehensive of the introduction of a new technology into an industry where, historically, hazards have surfaced only after serious harm was done to workers or the environment.

In the long run, the replacement of conventional chemical processing steps with biological processes should serve to reduce the level of overall risks. The microbes or enzymes that mediate the bioprocess will be susceptible to inactivation by high concentrations of many organic feedstocks. Thus, feed streams will have to be diluted with non-toxic substances to obtain concentrations that permit survival of the biological systems involved. As a consequence of this dilution, the feed streams will become less toxic to workers who run the processes and to an environment that may encounter the stream in the event of a spill.

All currently envisioned applications of biotechnology in the chemical industry anticipate the use of closed bioreactor systems for performing discrete chemical reactions or for growing large volumes of microorganisms for use as biocatalysts or as sources of substitute feedstocks. Experience accumulated in the pharmaceutical sector indicates that routine operation of such systems poses minimal environmental hazard. A typical fermentation operation is depicted in Figure 4-4. Each step in the process, including double-sealed stirring rotors, positive pressure inside the vessel with loss-of-pressure alarms to warn of a breach in containment, and pre-sterilization of all added materials, including air, anti-foaming agents, acid, and base for pH control, is conducted to ensure sterility and containment. Furthermore, since the air vented from the fermentor generates aerosols containing microorganisms, this exit gas should also be sterilized. Although not performed routinely, this can be accomplished by passing the air through high-efficiency particulate air (HEPA) filters, or by exposing the gas stream to radiation, electrical discharge, or germicidal sprays.

The fermentation process shown in Figure 4-4 involves sterilization of the reactor contents prior to sample work-up; that is, the microbes are killed before they are discharged from the vessel. The chemical industry might employ such a procedure in order to isolate an enzyme that the microbes have accumulated intracellularly or excreted into the medium. Figure 4-5 diagrams a process flow, including feed streams and waste streams, for isolation of an intracellular enzyme. The wastes from these processes consist of highly variable liquid streams containing high levels of suspended solids. These wastes typically have elevated chemical and biochemical oxygen demands (COD and BOD), as well as significant nitrogen and phosphate loadings. The pH is generally in the acceptable range, pH 5 to 9.

The application of biological processes in the chemical industry is in a very early developmental state. The ability of microorganisms, or their products, to mediate chemical transformations of organic substrates on a commercial scale has yet to be demonstrated. It seems probable that processes based on microbial systems will rely on activites that occur naturally among populations of microorganisms. Thus, genetic engineering to endow the microbe with new characteristics will find limited application for the foreseeable future; that is, for the next five to ten years.

Figure 4-4

Steps in a typical fermentation process

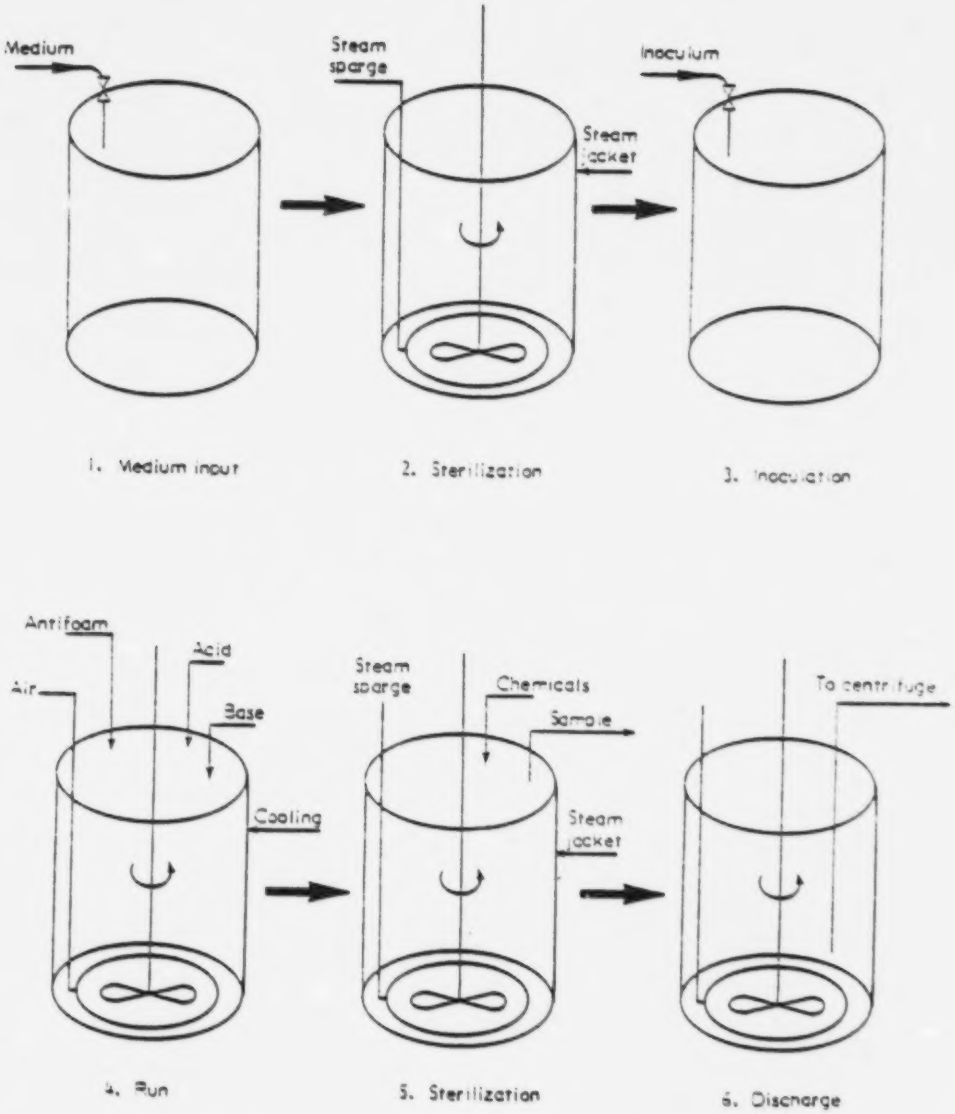
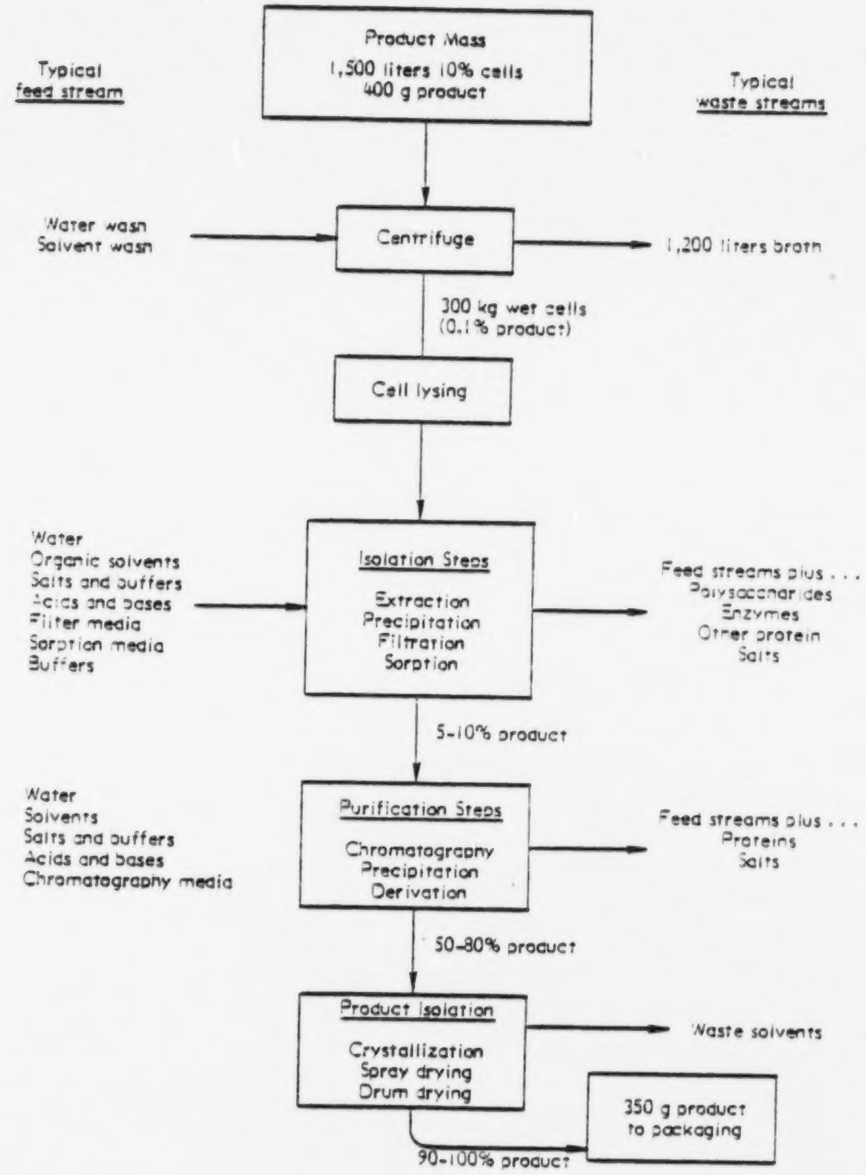

Medium
Steam sparge
Steam jacket
Inoculum
1. Medium input
2. Sterilization
3. Inoculation
Antifoam
Acid
Air
Base
Cooling
Steam sparge
Chemicals
Sample
Steam jacket
To centrifuge
4. Run
5. Sterilization
6. Discharge

Figure 4-5

Product recovery from a typical batch fermentation


Typical feed stream
Product Mass
1,500 liters 10% cells
400 g product
Typical waste streams
Water wash
Solvent wash
Centrifuge
1,200 liters broth
300 kg wet cells
(0.1% product)
Cell lysing
Water
Organic solvents
Salts and buffers
Acids and bases
Filter media
Sorption media
Buffers
Isolation Steps
Extraction
Precipitation
Filtration
Sorption
Feed streams plus . . .
Polysaccharides
Enzymes
Other protein
Salts
5-10% product
Water
Solvents
Salts and buffers
Acids and bases
Chromatography media
Purification Steps
Chromatography
Precipitation
Derivation
Feed streams plus . . .
Proteins
Salts
50-80% product
Product Isolation
Crystallization
Spray drying
Drum drying
Waste solvents
350 g product to packaging
90-100% product

## 4.3 Energy industry

### 4.3.1 Current activities

The potential applications of biotechnology in the energy field are vast. Proponents anticipate that a sizeable proportion of future world energy needs will be met through biological processes. Genetic engineering of microbes and higher plants will undoubtedly have significant impact on the development of future bioenergy systems, although activities to date have shown little evidence of this practice. Current activities in this industry will be considered within two general areas: energy from biomass and enhanced oil recovery.

#### 4.3.1.1 Energy from biomass

Biomass resources encompass all the storage repositories of solar energy. This includes photosynthetic organisms of all types, organisms that feed on photosynthetic biomass, and animal wastes. Biomass is a renewable energy source, a quality that distinguishes it from fossil fuels, which are also derived from biomass, but which require eons of time to develop. The energy content of the carbohydrates generated annually in higher plants alone has been estimated to be **ten** times the global energy consumption. The inclusion of marine biomass, such as phytoplankton, might increase this factor another ten-fold. Clearly, tapping this vast energy supply must be considered a top priority in the years ahead.

Biomass fuel sources have five major advantages over fossil fuels:

- They are renewable;
- They do not contribute to carbon dioxide pollution because, at a steady state, carbon dioxide is incorporated into plant material and removed from the atmosphere at the same rate that it is put into the atmosphere by combustion;

- The rate of carbon dioxide fixation into usable plant material by photosynthesis is fifty times greater than our current rate of fossil fuel consumption;
- Biomass potential is more evenly distributed geographically than are fossil fuel reserves; and,
- The potential market in biomass is huge allowing R&D costs to be amortized over a large number of production units.

Biological systems useful in the conversion of biomass to liquid fuel have not been intensely developed. Current commercial practice is founded on the production of alcohol for distilled beverages. Corn is the main feedstock and the yeast Saccharomyces cerevisiae is the principal fermentation organism. It is clear that S. cerevisiae can be made to convert carbohydrates by fermentation to ethanol with a much higher efficiency than is currently achieved. This higher yield can be approached in two ways: (l) a greater mass of ethanol can be produced per mass of carbohydrate consumed, and (2) a product with a higher percentage of ethanol can be produced. The overall efficiency of the process can be improved by exploring mixed bacterial-yeast fermentation systems and by adapting the whole fermentation process to a continuous flow mode.

**Ethanol** for use as fuel, either alone or mixed with gasoline to make gasohol, can be produced by microbial fermentation of sugars. Two sources of sugars abound. First, **starch** (for which fermentation technology is well advanced) is available from edible plant products, such as corn, wheat, potatoes, sugar cane, sugar beets, and cassava. Second, **cellulose,** from which conversion to ethanol is difficult, is abundant in municipal/agricultural wastes and forests. Utilizing starch as the feedstock for ethanol production entails a diversion of crop land that could otherwise contribute to the food supply. This disadvantage has not deterred the government of Brazil from investing $5 billion during the past decade on facilities to manufacture ethanol from cassava, sugar, and molasses. Ultimately, all of Brazil's motor vehicles will be run on ethanol. It is estimated that 2% of the nation's land will be devoted to this enterprise.

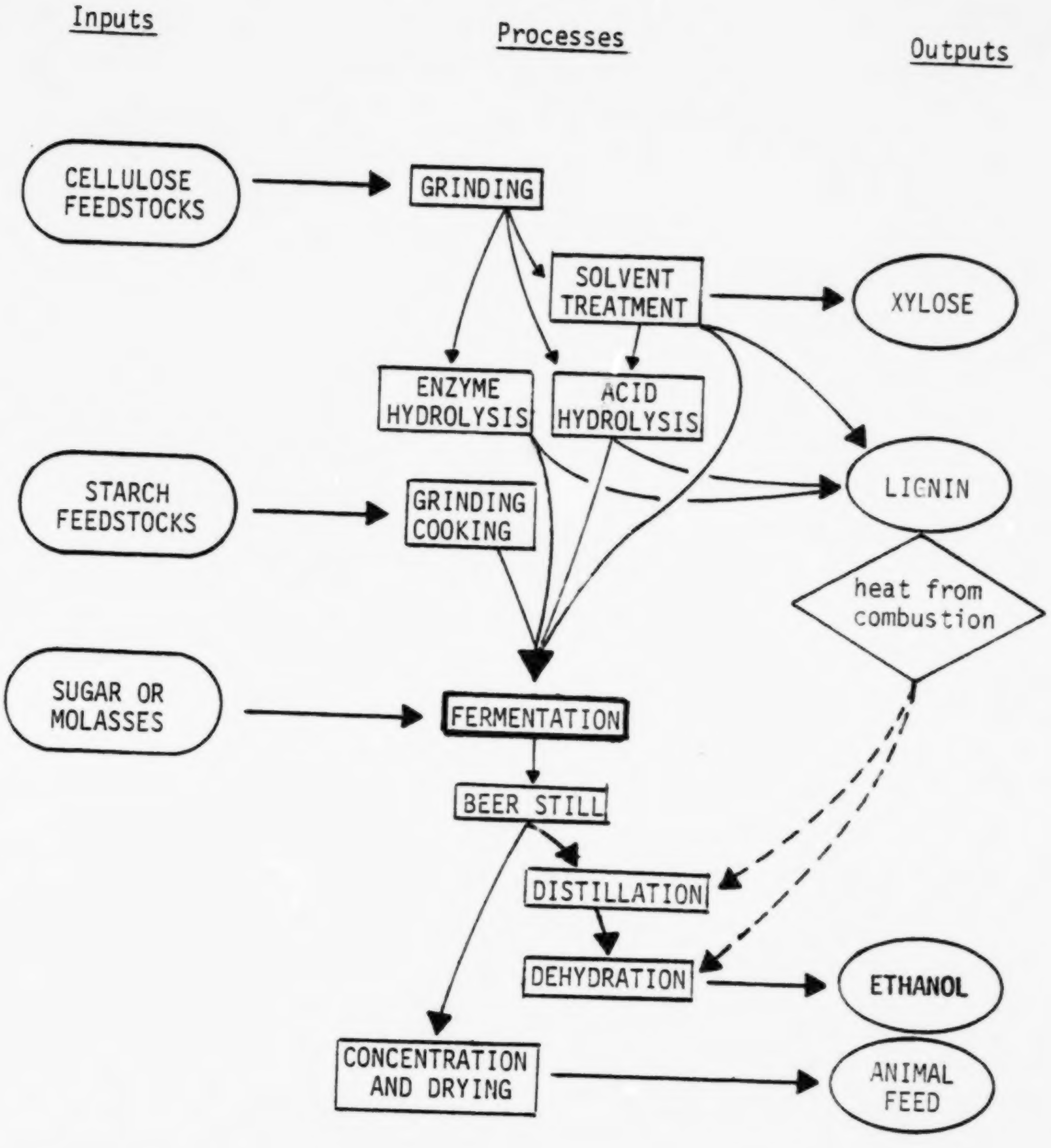
Ethanol represents one of the most promising alternative fuels to OPEC oil. It can be burned in conventional automobile engines without modification as a 20% alcohol/80% gasoline mixture. Relatively minor engine and fuel system adjustments are required to convert gasoline engines to 100% ethanol use. The Ford Motor Co. of Brazil currently sells a conversion kit for about $250 that will convert a standard auto engine to permit use of 100% ethanol. Alcohol can be efficiently handled as a fuel by existing petroleum distribution networks.

Ethanol production by yeast may be greatly enhanced using molecular cloning techniques. The biochemical pathway unique to ethanol metabolism is relatively simple. Pyruvate is converted by the enzyme pyruvate decarboxylase to acetaldehyde and carbon dioxide. Acetaldehyde is converted to ethanol by the enzyme alcohol dehydrogenase. The gene for alcohol dehydrogenase has been cloned in several laboratories and it appears possible to increase the efficiency of the fermentation process by increasing the level of alcohol dehydrogenase in the cell using genetic engineering techniques. The other enzyme in the process, pyruvate decarboxylase, should also be amenable to genetic engineering.

In the long run, ethanol production from cellulosic wastes will be preferable to using foodstuffs as the raw material. Typical cellulosic materials consist of 50% cellulose (a glucose polymer), 25% hemicellulose (a polymer of xylose, a five-carbon sugar), and 25% lignin (a complex phenolic polymer). This semi-crystalline **lignocellulose** is broken down with difficulty into fermentable constituents, by acid treatment or by the enzyme cellulase. This expensive initial phase of cellulose preparation is where process improvements are most needed. Figure 4-6 provides a general scheme for ethanol production utilizing either cellulosic or starch feedstocks, and Figure 4-7 provides an overview of the variety of petrochemical feedstocks that can be obtained from cellulosic starting materials.

Figure 4-6

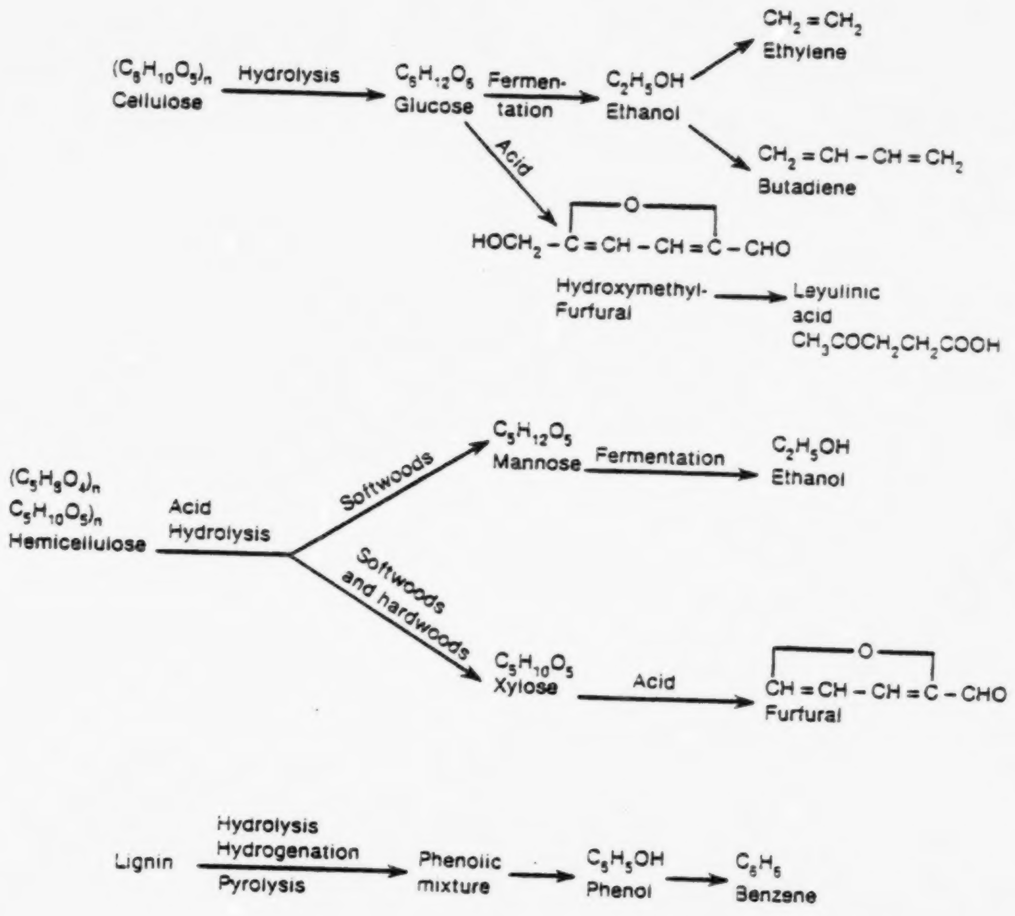
Steps in the conversion of biomass to ethanol and by-products


Inputs
Processes
Outputs
CELLULOSE FEEDSTOCKS
GRINDING
SOLVENT TREATMENT
XYLOSE
ENZYME HYDROLYSIS
ACID HYDROLYSIS
LIGNIN
STARCH FEEDSTOCKS
GRINDING COOKING
heat from combustion
SUGAR OR MOLASSES
FERMENTATION
BEER STILL
DISTILLATION
DEHYDRATION
ETHANOL
CONCENTRATION AND DRYING
ANIMAL FEED

Source: King, S.R. (1979)

Figure 4-7

The conversion of lignocellulose into useful chemical feedstocks


(C6H10O5)n
Cellulose
Hydrolysis
C6H12O6
Glucose
Fermen-
tation
C2H5OH
Ethanol
CH2 = CH2
Ethylene
CH2 = CH - CH = CH2
Butadiene
Acid
HOCH2 - C = CH - CH = C - CHO
O
Hydroxymethyl-
Furfural
Levulinic
acid
CH3COCH2CH2COOH
(C5H8O4)n
C5H10O5)n
Hemicellulose
Acid
Hydrolysis
Softwoods
C6H12O6
Mannose
Fermentation
C2H5OH
Ethanol
Softwoods
and hardwoods
C5H10O5
Xylose
Acid
CH = CH - CH = C - CHO
O
Furfural
Lignin
Hydrolysis
Hydrogenation
Pyrolysis
Phenolic
mixture
C6H5OH
Phenol
C6H6
Benzene

**Methane** generation by anaerobic digestion of biomass provides another route whereby renewable resources are utilized for energy production. Animal feedlot wastes and municipal sewage are most often cited as providing the raw materials for this process, although forest residues and food crop biomass are also suitable. The process produces:

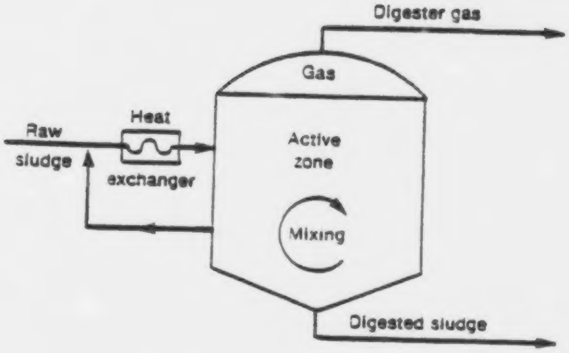
- Biogas, consisting of approximately 60% methane and 40% carbon dioxide;
- Residual solids, containing vegetable proteins, which have potential value as feed additives or fertilizers; and,
- Spent process water, laden with nutrients, which is suitable for growing algae or as a fertilizer.

Since the process occurs in closed digesters to exclude oxygen, the waste materials used as feedstock are prevented from spoiling the environment or giving rise to pathogenic organisms. Anaerobic reactors are classified into three types depending on the operating temperature (i.e., the optimal temperature for growth of the particular microbial strain involved): (1) **psychophilic** (under 20°C), (2) **mesophilic** (20° to 45°C), and (3) **thermophilic** (45° to 65°C). A typical digester used for sewage treatment is depicted in Figure 4-8.

Most applications of this technology involve small, community-scale operations. Biogas generators associated with large animal feedlots or municipal sewage treatment facilities might readily supply the energy needs of the local population. Simple anaerobic digesters of this type are common in the People's Republic of China, Korea, Taiwan, and India. But large-scale operations may be feasible. A study commissioned by the U.S. Science and Education Administration of the USDA found that economical biogas production could be achieved with feedlots averaging 1,000-2,000 head of cattle in size. Others have proposed a large, centralized facility that could produce 50 million cubic feet of methane per day using biomass crops as feedstock. Also, the mass-cultivation of water

Figure 4-8

A single-tank anaerobic digester of biogas production


Digester gas
Gas
Raw
sludge
Heat
exchanger
Active
zone
Mixing
Digested sludge

Source: U.S. Environmental Protection Agency (1979) hyacinths on sewage lagoons for use as a fermentation substrate has been proposed. Current R&D efforts in this area tend to emphasize aspects of process design and engineering rather than microbiology. Nevertheless, genetic engineering may have a significant role in future developments of biogas production.

**Hydrocarbons** are synthesized and accumulated by a wide variety of bacteria, algae, and yeasts (see Table 4-6). These microbes generally extract carbon dioxide from air and utilize energy derived from photosynthesis to reduce chemically and polymerize $CO_2$ into long-chain lipids. Some microbes utilize carbohydrates such as glucose as carbon sources. As much as 40-50% of the dry weight of certain oil-bearing microorganisms can consist of reduced hydrocarbon materials suitable as substitute fuels. Many higher plant species produce a sap or fruiting body that is high in hydrocarbon content. Most familiar are vegetable oils, such as sunflower, cottonseed, linseed, palm, etc., some of which are under investigation as diesel fuel additives. A variety of less familiar tropical plants and trees is also under examination as hydrocarbon producers.

The production of **hydrogen** gas from water has been demonstrated in laboratory studies—its commercial-scale feasibility remains to be shown. The system utilizes units of photosynthetic activity, called **chloroplasts**, isolated from green plants, such as lettuce or spinach. A biophotolysis reaction is established in which energy from sunlight splits water ($H_2O$) into molecular hydrogen ($H_2$) and oxygen ($O_2$). Successful operation of the system requires a means of removing oxygen to prevent reaction with hydrogen to regenerate water. Rather than isolating chloroplasts from higher plants, it may be preferable to use intact, photosynthetic algae or bacteria. The future use of hydrogen as a fuel offers the promise of a non-polluting, inexhaustible energy source. However, numerous technical obstacles remain before this prospect will be realized.

## Table 4-6

## Some species of algae that produce hydrocarbons

| **Species** | **Lipid content (% dry wt.)** |
|---|---|
| *Biddulphia aurita* | 12.2 |
| *Chlamydomonas applanate* | 32.8 |
| *Chlorella pyrenoidosa* | 14.4 |
| *Chlorella vulgaris* | 28.8 |
| *Monallanthus salina* | 40.8 |
| *Nannochloris* sp. | 20.2 |
| *Nitzschia palea* | 22.2 |
| *Oocystis polymorpha* | 12.6 |
| *Ourococcus* sp. | 27.0 |
| *Skeletonema costatum* | 23.8 |

Source: Shifrin, N.S. and Chisholm, S.W. (1980) in "Algae Biomass", p. 633, Elsevier Press.

### 4.3.1.2 Enhanced oil recovery

A second major area of the energy field in which applied genetics will have an impact is **not** the creation of new sources of energy but enhanced recovery from existing energy supplies. Primary and secondary oil recovery techniques manage to extract only about one-half of a known oil reservoir. An estimated 200 billion barrels of oil in the continental United States remain out of reach with conventional recovery techniques. A variety of microbial-based tertiary recovery methods has been proposed as a means to tap this vast resource. These include:

- The injection of oil-degrading bacteria into an oil field would reduce the oil's viscosity, or convert oil to natural gas;
- The injection of microbes to re-pressurize a spent oil well by synthesizing carbon dioxide or other gaseous metabolite;
- The injection of microbes that manufacture and secrete chemical surfactants that would act to mobilize tightly bound oil.

In addition to these potential applications in existing oil fields, microbial processes have been promoted for use in extracting tar and oil (bitumen) lodged in tar sands. Also, a bacterial process is under development at the University of Southern California that would release kerogen (a petroleum material) from oil shale. This process could generate a barrel of oil per ton of western oil shale without extensive ore crushing, retorting, or environmental damage that attend strictly physical recovery methods. Finally, analysis of subsoil microbial populations may assist in locating previously unknown oil and gas fields below. This microbiological method of prospecting for petroleum is still in an early stage of commercial development, as are all of the microbial recovery methods described above.

### 4.3.2 Future prospects

The range of potential uses of applied genetics in the energy industry appears to be far wider than in the chemicals sector. However, most of these possibilities lie far in the future, at least with regard to large-scale commercial application. Development of systems for ethanol production from biomass for use in gasohol are proceeding apace, especially in petroleum-poor areas like Brazil, but the economics of this process and the energy savings incurred will remain unfavorable, probably for the remainder of the 1980's. Nevertheless, several long-range projects can be envisioned that may one day provide significant sources of energy.

- Mass production of hydrocarbon substances from various species of higher plants, including those listed in Table 4-4, can become economically feasible when either (1) plant cells are manipulated to grow in massive cultivators, akin to microbial fermentors, in which excreted hydrocarbons are continuously collected, or (2) the genetic information that enables the plant cell to synthesize hydrocarbons is transferred to microorganisms which, in turn, manufacture and excrete the fuel-like substances. The biotechnical and engineering obstacles that stand in the way of such a project are formidable.

- A biological solar battery will someday replace the panels of silicon solar cells that find specialized uses today. The biological battery will operate via a direct conversion of sunlight into electricity (i.e., a current of electrons) that is generated during photosynthesis. Although all green plants engage in photosynthesis and are, therefore, suitable sources of materials for constructing a biological battery, a primitive, purple photosynthetic bacterium, called Rhodospirillum rubrum, may be exploited as the living solar cell. Alternatively, the photsynthetic blue-green algae, which utilize carbon dioxide and nitrogen directly from air may serve this purpose.

- Ethanol production may become more efficient through use of microorganisms other than common yeasts (e.g., Saccharomyces cerevisiae, or brewer's yeast). A bacterial species, called Zymomonas mobilis, carries out alcoholic fermentation two to three times faster than yeasts. This bacterium, now employed to make tequila, is under investigation by researchers at the USDA's Northern Regional Research Lab in Peoria, Illinois.

- Acidophilic, iron-oxidizing Thiobacilli bacteria (commonly used in mineral leaching operations, see Section 4.4.1) may prove useful in oil shale or coal conversion processes. The bacteria will mobilize the inorganic mineral content of the shale or coal without affecting the hydrocarbon content of the material. The porous zones that this process generates in situ may assist in subsequent retorting or gasification schemes.

### 4.3.3 Potential hazards

The application of biological processes to the energy industry is at a very early stage of development. Other than the fermentation of ethanol from cornstarch for use in gasohol production, no commercial-scale bioprocess will have an impact on the energy sector for at least five years. The production of biofuels (e.g., ethanol, methane, vegetable hydrocarbons) from unconventional feedstocks has progressed only to the pilot scale, whereas biological hydrogen production is little more than a laboratory curiosity at present. Likewise, field tests have so far failed to demonstrate the general feasibility of using microbial systems for enhanced oil recovery. Thus, potential environmental hazards resulting from the use of applied genetics in energy production are highly speculative. Nevertheless, several comments are appropriate and some areas of potential concern can be identified.

- The production in the United States of sufficient ethanol to have a significant impact on domestic fuel supplies will require the diversion of enormous quantities of food crops, particularly corn. According to one estimate, a 4 billion gallon-per-year ethanol program could result in a 10 to 20% shortfall in corn supplies by 1990. This would severely limit the availability of grain for livestock feed or exports, thereby driving up food prices. Four billion gallons represent less than 5% of current annual fuel consumption. Clearly, useful alternative biomass feedstocks for ethanol production are sorely needed.

- The prospect that cellulosic materials may serve as suitable feedstocks for biofuel production forewarns of large-scale deforestation, particularly in areas lacking alternative sources of biomass. Huge tracts of prime forest land in certain parts of the world have already been cleared for purposes of agriculture or fuel use. The wholesale conversion of wood biomass into ethanol threatens to exacerbate this trend.

- Processes designed to convert lignocellulosic materials into substrates suitable for ethanol fermentation entail an initial hydrolysis step (see Figure 4-6). Hydrolysis can be accomplished either chemically, using strong mineral acids, or biologically with enzymes. The latter approach is preferable from a safety and environmental point of view but is less likely to be implemented in the near term. Thus, commercial processes generating large quantities of acid wastes can be anticipated.

- As mentioned previously, the utilization of wastes and municipal sewage as raw materials for biogas generation promises to lessen the environmental burden imposed by these pollutants. Hazards may arise, however. If large centralized biogas facilities are planned, then one faces risks associated with the transport of the raw wastes to the site from various points of origin. A program to establish numerous local biogas generating stations may encounter variations in operating characteristics or in the level of personnel training that could mitigate against long-term safe operation of any particular facility.

- The species of microorganisms likely to be utilized in enhanced oil recovery schemes -- Pseudomonas and Acinetobacter, for example -- are the same as those mentioned previously in the context of biotransformations of organic substances in the chemical industry. As already discussed, these microbes are potentially serious pathogens in man.

- It is probable that the near-term use of microorganisms to mediate bioprocesses in the energy industry will exploit naturally occurring microbes. Thus, as is true for the chemical industry, the impact of genetic engineering (especially recombinant DNA techniques) will be minimal.

- Finally, established energy companies are not accustomed to dealing with biological systems as a means of producing energy. (Most oil companies, however, do maintain some expertise in microbiology to assist in prospecting.) These firms will be compelled to strengthen their technical competence in areas related to biology as commercial prospects for bioenergy brighten. Hopefully, they will devote adequate attention to environmental hazards that may emerge from these new areas of business.

## 4.4 Mining industry

### 4.4.1 Current activities

The impact of biotechnology on the mining industry is currently quite limited in scope, consisting of two general areas:

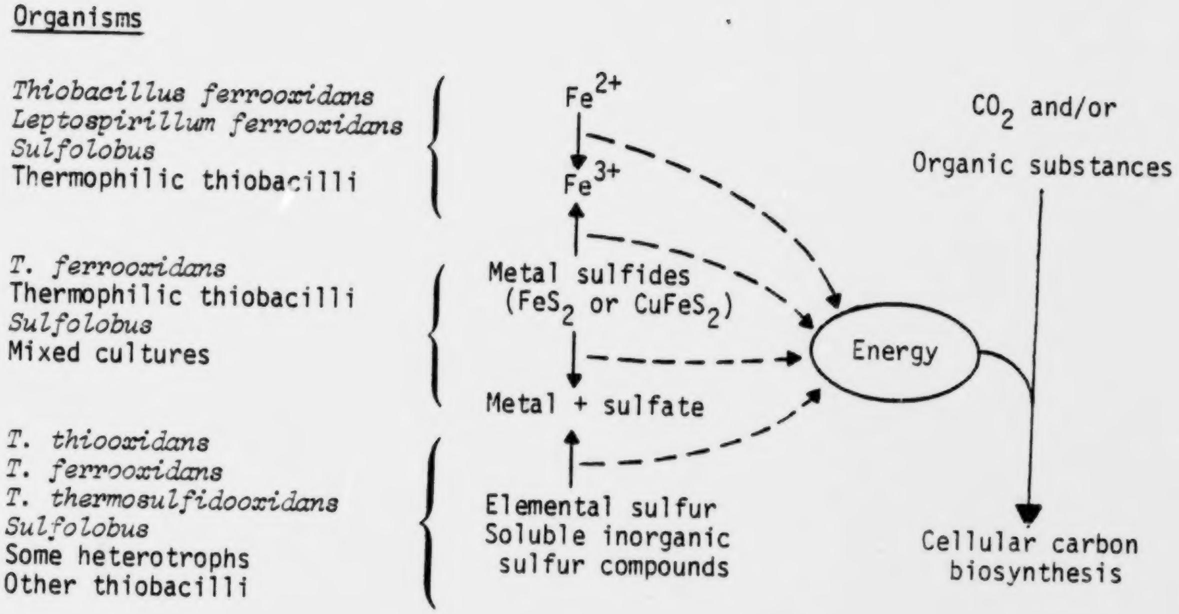
- The **accumulation** of metals by organisms, either by binding at cell surfaces or by intracellular uptake of metals; and,
- Biochemical **transformations** of metals, including solubilization or precipitation, oxidation/reduction processes, and the interconversion of inorganic and organic metal compounds.

The various bioprocesses subtended under these categories are all carried out by a relatively small number of bacterial species. Figure 4-9 lists these organisms and summarizes the means by which these microbes extract energy from chemically reduced inorganic compounds (such as ferrous iron or sulfur compounds) and employ either inorganic ($CO_2$) or organic carbon sources.

The microbial process whereby metals are solubilized from their ores is called **bacterial leaching.** The operation consists of percolating acidified water through heaps or dumps of low-grade ore that may contain up to four billion tons of rock. Bacterial action within the dump oxidizes mineral sulfide, producing sulfuric acid, and solubilizes the metal. The solution, or leachate, is collected and processed to recover the dissolved metal. The residual liquid, containing sulfuric acid and ferrous/ferric iron, is recycled to the dump. This somewhat crude process has been used in mining operations since Roman times. Currently, its greatest use occurs in copper and uranium mining operations. Approximately 12% of U.S. copper production stems from dump leaching of this type.

Figure 4-9

Leaching bacteria: organisms and basic metabolism


Organisms
*Thiobacillus ferrooxidans*
*Leptospirillum ferrooxidans*
*Sulfolobus*
Thermophilic thiobacilli
*T. ferrooxidans*
Thermophilic thiobacilli
*Sulfolobus*
Mixed cultures
*T. thiooxidans*
*T. ferrooxidans*
*T. thermosulfidooxidans*
*Sulfolobus*
Some heterotrophs
Other thiobacilli
$Fe^{2+}$
$Fe^{3+}$
Metal sulfides
($FeS_2$ or $CuFeS_2$)
Metal + sulfate
Elemental sulfur
Soluble inorganic
sulfur compounds
Energy
$CO_2$ and/or
Organic substances
Cellular carbon
biosynthesis

Source: Bull, A.T., etal. (1979)

The feasibility of large-scale dump leaching was first demonstrated in 1750 in Rio Tinto, Spain. The technology is still practiced widely in mining operations throughout the western United States, including mines owned by Kennecott Copper at Bingham Canyon, Utah and Santa Rita, New Mexico, as well as the Butte, Montana mines operated by Anaconda Copper.

Despite the long history of mineral leaching, the role of microorganisms as mediators of the process was not recognized until the mid-1950's. The principal microbes involved in copper extraction are Thiobacillus ferrooxidans and Thiobacillus thiooxidans. Both species are rod-shaped, aerobic bacteria that thrive in an acid environment (pH 1.5 to 3.0) and use carbon dioxide as a carbon source. They function within a temperature range of 18° to 40°C (64° to 104°F).

The bacteria require, in addition to water and oxygen, a reduced iron or sulfur energy source, as seen in the following equations (unbalanced):

$$Fe^{II}SO_4 + O_2 + H_2SO_4 \longrightarrow Fe_2^{III}(SO_4)_3 + H_2O$$

$$S_8 + O_2 + H_2O \longrightarrow H_2SO_4$$

$$H_2S + O_2 \longrightarrow H_2SO_4$$

Ferric iron ($Fe^{III}$) and sulfuric acid ($H_2SO_4$) generated by these bacterial reactions are very effective chemical solubilizers for numerous minerals, including those listed in Table 4-7. In addition to those shown, other minerals, such as uranium oxides, that co-exist with iron or sulfur-containing ores, are readily leached. These reactions occur at rates approximately 500,000-fold faster than the oxidation of iron and sulfur by air in the absence of bacteria. Both of the Thiobacillus species are found in great abundance in leaching operations--as many as $10^7$ organisms per gram of ore. Indeed, the high concentration of microbes in leachate solutions poses difficulties during subsequent mineral extraction and isolation.

## Table 4-7

## Minerals readily leached by bacterial action

| Mineral | Formula |
|---|---|
| pyrite | $FeS_2$ |
| chalcopyrite | $CuFeS_2$ |
| chalcocite | $Cu_2S$ |
| covellite | $CuS$ |
| arsenopyrite | $AsFeS$ |
| molybdenite | $MoS_2$ |
| stibnite | $Sb_2S_3$ |
| pentlandite | $NiFeS_2$ |
| zincblende | $ZnS$ |

Bacterial leaching is also utilized to recover uranium from low-grade ores, mine tailings, and other ores that are rich in pyrite. The following reaction pertains to this process:

$$UO_2 + Fe_2^{III}(SO_4)_3 \longrightarrow UO_2SO_4 + Fe^{II}SO_4$$

This solubilization process has been used in scavenger operations in mined-out and low-grade stopes in the Elliot Lake region of Ontario. The ores of northern Ontario are amenable to bacterial leaching due to the presence of large amounts of pyrite, whereas the uranium deposits in the U.S. Rocky Mountains and southern Texas contain insufficient pyrite to allow successful leaching operations.

Other bacterial species have been implicated in mineral leaching, including some members of the *Sulfolobus* genus. These bacteria are obligate thermophiles, requiring a temperature range of 45° to 80°C (110 to 175°F), as well as an acidic environment. *Leptospirillum ferrooxidans* is another iron-oxidizing acidophile that has been shown to release pyrite more efficiently than *T. ferrooxidans* when grown in mixed cultures with sulfur-oxidizing bacteria.

All organisms, including microbes, can accumulate certain metal ions that are essential for metabolic activity. Iron, magnesium, zinc, manganese, copper, cobalt, nickel, moybdenum, and vanadium are required by various organisms, albeit frequently in trace quantities only. Nevertheless, certain microbes have evolved highly efficient means of permitting the selective concentration of metals far in excess of the local concentration. Toxic metals, such as cadmium, lead, silver, and thallium, can be accumulated even though these substances have no metabolic function. Apart from the intracellular uptake of metals, positively charged metal ions can be removed from solution by adsorption onto the negatively charged surface of the microbe.

Microorganisms can also be utilized in the restoration of wastewaters from mining and milling operations. One successful operation uses algae to remove both soluble and particualte lead from the mill tailings of several mining ventures in Missouri.

These operations consist of settling ponds and a series of shallow meanders in which the algae are encouraged to grow. Chemical analysis has shown that the algae accumulate heavy metals from the effluent released from the settling pond. Algae species that have been identified to function effectively in these types of operations include: Cladophora, Rhizoclonium, Hydrodictyon, Spirogyra, Potamogeton, and Oscillatoria.

At present the potential to use genetic engineering to improve the performance of these, or other, algae species is remote, and may not be possible for several years. On the other hand, genetic engineering techniques could certainly be used to improve Thiobacillus ferrooxidans.

As explained above, T. ferrooxidans derives its energy from the oxidation of ferrous ion, metal sulfides, and soluble sulfur compounds in an acidic medium. The ferric ion generated in the form of ferric sulfate is then able to react chemically with several ore minerals and oxidize them. The ferric ion is then regenerated by the microorganisms. Apparently, one of the primary rate-limiting steps in the leaching of metal ores is the ferrous-to-ferric reoxidation. Ferric ion competitively inhibits the rate of ferrous ion oxidation. Thus, as the concentration of ferric ion increases, its production is slowed.

Since ferric ion has no other metabolic effect on T. ferrooxidans except to slow its own production, it should be straightforward to isolate a suitable mutant strain that is not affected by ferric ion concentration.

Thiobacilli are able to develop considerable resistance to the very high concentrations of the metals being leached, but the microbe is inhibited by some metals such as silver, mercury, and cadmium, at quite low concentrations. Metabolic resistance to heavy metals is frequently conferred by the presence of certain bacterial plasmids. Experiments could be undertaken to isolate appropriate plasmids from other bacteria and to introduce them into Thiobacilli using recombinant DNA or conventional genetic technologies.

Improved bacterial growth and mineral leaching activity have resulted when Thiobacilli are grown in conjunction with the nitrogen-fixing bacterium, Beijerinckia lacticogenes. This latter bacterial species probably supplies Thiobacilli with essential nitrogenous nutrients. Since B. lacticogenes is less able to withstand the highly acidic environment required by Thiobacilli, it may prove worthwhile to introduce the nitrogen fixation genes (nif genes) directly into Thiobacilli. Alternatively, nif genes from Azotobacter or Klebsiella (two free-living nitrogen fixers) can be utilized since these species share several structural and biochemical features with Thiobacilli.

### 4.4.2 Future prospects

Of the five industrial sectors considered in this report, the mining industry has demonstrated the least interest in applying biotechnology to its operations. The types of bioprocesses that **do** pertain to mining are rather limited in scope, but technical advances, leading to increased interest on the part of the industry, can be envisioned.

- All known strains of leaching bacteria are aerobic; that is, they require oxygen. However, essentially oxygen-free conditions exist in the center of huge slag heaps of low-grade ore. Thus, the engineering of anaerobic strains of Thiobacillus would be received with great enthusiasm by the mining industry. The technical feasibility of this proposal is uncertain. Likewise, development of improved thermophilic leaching bacteria would be very useful, owing to the heat generated within ore dumps.

- The United States relies on imports for the vast majority of certain mineral resources, including chromium, titanium, and manganese (see Table 4-8). Recycling of these materials is of increasing importance. The development of efficient microbiological systems for extracting these metals from industrial effluents and other waste repositories would constitute a major industrial and political tour de force.

- Very little basic information is available regarding the biochemistry and genetics of leaching bacteria. Consequently, genetic engineering, especially recombinant DNA, will have little impact on developments in this area for at least five years. The properties and commercial suitabilities of existing, naturally occurring leaching bacteria will undergo thorough examination first.

### 4.4.3 Potential hazards

The limited scope of biotechnology in the mining industry confines the range of environmental concerns that demand consideration. However, all foreseeable applications of biological processes in this industry involve microbial systems operating in relatively open environments, such as slag heaps or tailings ponds. Consequently, there are risks that microorganisms or their metabolic products will inadvertently contaminate the local ecology. Specific areas of concern include the following.

- Bacterial leaching operations generate large quantities of sulfuric acid which, if poorly contained, could seriously contribute to the acidification of U.S. fresh water supplies.

- Thiobacilli and related bacterial species are not known to be pathogenic in man or animals; indeed, their peculiar metabolic characteristics suggest that they should be quite innocuous from a public health standpoint. However, increased use of such microbes on an industrial scale (resulting in greater exposure to human populations) may select for bacterial strains that have acquired the ability to infect humans.

- The use of bacteria to concentrate metals from dilute waste streams or settling pond entails the risk that metals will accumulate in the food chain. Even though metal ions, such as mercury and silver, are highly toxic to bacteria, it is through microbial action that mercury, for example, is transformed into organic compounds that are responsible for mercury toxicity in higher forms of life. In other words, metals released into the environment are metabolized by naturally occurring bacteria. The key for safe commercialization of this bioprocess will be adequate **containment** of the operation to prevent dissemination of toxic metals into the general environment.

- As with other industrial sectors examined in this report, the mining industry has very little experience with biological processes. This lack of familiarity could result in a failure to recognize impending environmental hazards or in an eagerness to carry out biological processes before their safety has been firmly established.

### Table 4-8

### Strategic minerals and U.S. dependence on foreign sources

| Mineral | Uses | Percentage imported | Sources |
|---|---|---|---|
| bauxite | aluminum | 94% | Jamaica, Guinea, Surinam |
| chromium | ferroalloys | 91 | South Africa, USSR |
| cobalt | superalloys | 93 | Zaire, Belgium, Zambia |
| columbium | ferroalloys | 100 | Brazil |
| manganese | steel | 97 | Gabon, South Africa |
| nickel | steel | 73 | Canada |
| platinum | catalysts | 87 | South Africa, USSR |
| rutile | pigments | 100 | Australia |
| tantalum | electronics components | 97 | Thailand |
| titanium | aerospace components | 47 | Japan, USSR |

## 4.5 Pollution control industry

### 4.5.1 Current activities

The organic matter invested in all living things, whether plant or animal, is eventually recycled back into the environment as $CO_2$. The process whereby organic carbon is converted into inorganic carbon is called **mineralization.** Representing a major portion of the overall **carbon cycle** (see Figure 4-10), mineralization is almost always a consequence of microbial action. That is, bacterial decomposers are ultimately responsbile for the degradation of all organic carbon-containing substances in the biosphere. For example, bacteria of the *Pseudomonas* species metabolize simple alkane compounds, such as octane, by means of an enzymatic oxidation pathway that converts the alkane ($R-CH_3$) into the corresponding carboxylic acid (R-COOH). The acid is then consumed as an energy source by the bacterium via further oxidation to carbon dioxide.

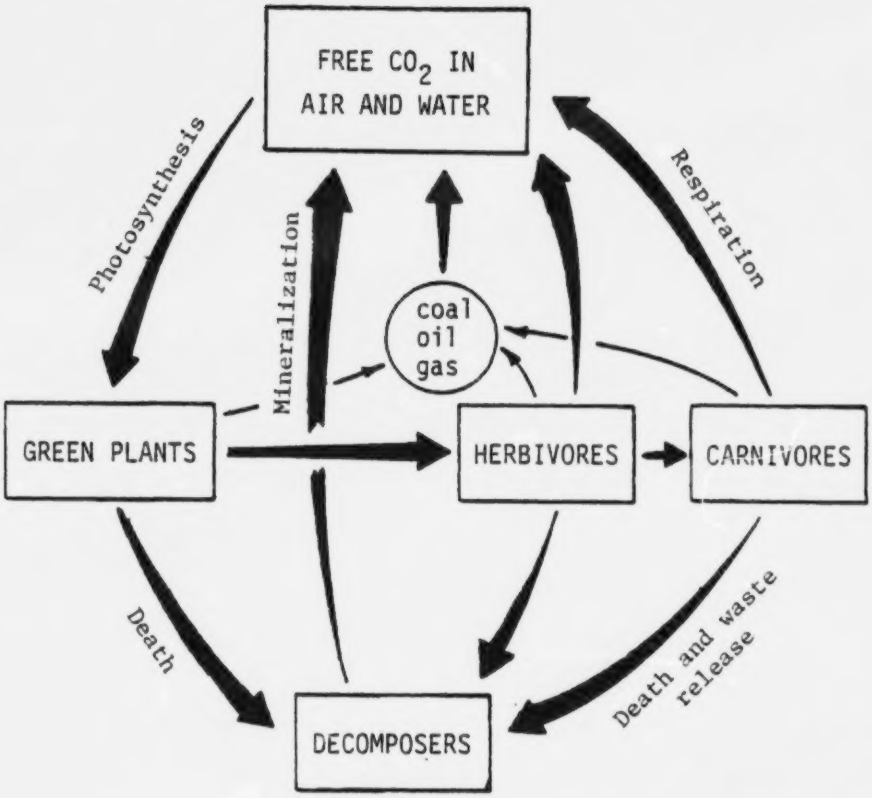
Therefore, it is hardly surprising that microbiology plays an important role in pollution control and waste management, particularly in the case of organic pollutants. Moreover, inorganic pollutants, such as nitrogen-containing substances and toxic metals, are often treatable using biological systems. Biological waste management has been practiced by mankind literally for thousands of years, but modern advances in applied genetics may revolutionize the pollution control industry to the extent that bioprocesses may soon replace many currently employed chemical/physical systems. Current activities within this industry fall under three general headings:

- Biodegradation of organic substances, such as petroleum products, pesticides, herbicides, industrial solvents, and lignin wastes;
- Biological denitrification and desulfurization processes; and,
- Removal or concentration of toxic heavy metals.

Each of these areas will be examined in the following sections.

Figure 4-10

The carbon cycle


FREE $CO_2$ IN AIR AND WATER
Photosynthesis
Mineralization
Respiration
coal oil gas
GREEN PLANTS
HERBIVORES
CARNIVORES
Death
Death and waste release
DECOMPOSERS

### 4.5.1.1 Biodegradation of organic substances

Most current activity, and that which has the most potential for biological waste treatment, exploits the capacity of microbes to degrade toxic chemical pollutants. A great variety of naturally occurring microorganisms, largely isolated from soil or aquatic environments, are known to utilize hazardous organic substances as carbon and energy sources. Table 4-9 provides a sample of the biodegradative processes that are currently in use or under investigation. Figure 4-11 shows the degradative pathways of several specific pollutants.

Efforts to improve on nature by applications of genetic engineering in this area have been minimal to date, but such activities are certain to increase in the near future. Several efforts bear mentioning. Among the first highly publicized uses of genetic engineering was that of Chakrabarty, then at General Electric. He combined the qualities of several strains of the bacterium Pseudomonas, each of which could degrade a single hydrocarbon component of crude oil, into a single bacterial strain. This "man-made" bacterial culture proved superior to a mixed microbial culture composed of each of the contributing strains in breaking down crude petroleum. The "oil-eating" microbes feed on the crude petroleum, converting the hydrocarbon compounds into cellular constituents (biomass) and carbon dioxide. However, the petroleum constituents that are converted by this microbial strain (namely camphor, naphthalene, and short-chain alkanes) are not the major environmental concerns arising from oil spills. These volatile hydrocarbons are either vaporized or readily degraded by natural bacterial action. Of greater impact are the various asphaltenes that constitute the heavy, non-volatile fraction of crude oil. These compounds are extremely refractory to microbial degradation. Despite the publicity that attended Chakrabarty's efforts, GE has not pursued this project beyond the laboratory stage of development.

Scientists at the Battelle Memorial Institute in Columbus, Ohio, are engaged in genetic engineering of microbes that efficiently degrade the chlorinated

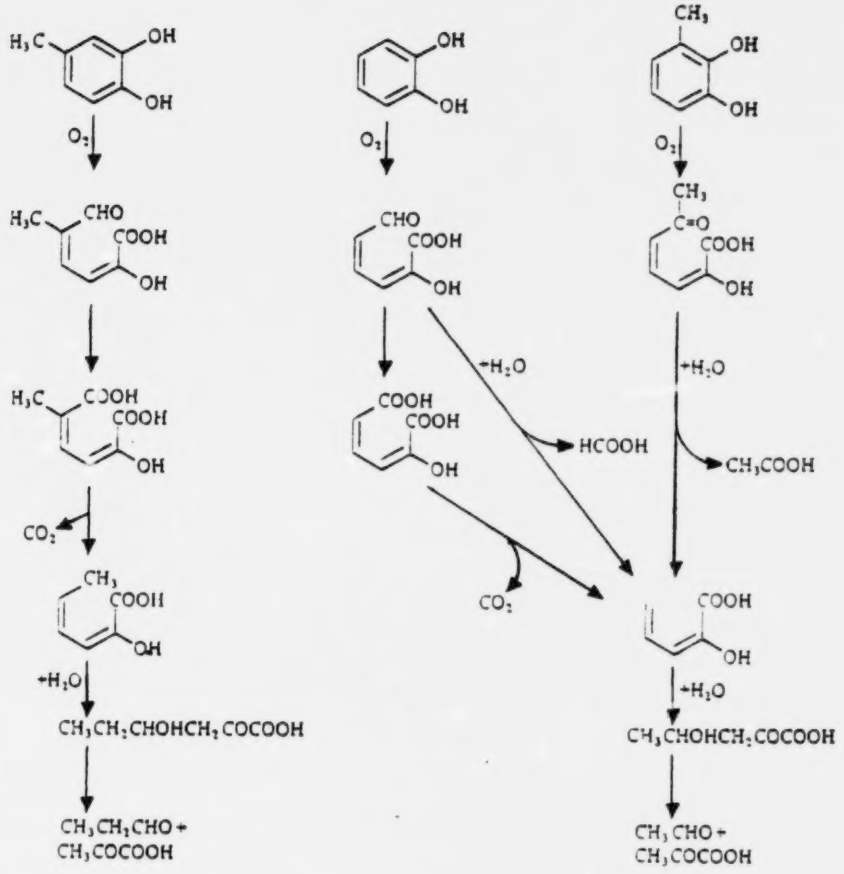
## Table 4-9

## Microbial degradation of various organic pollutants

| Pollutant | Microbes involved |
|---|---|
| **I. petroleum hydrocarbons** | 200+ species of bacteria, yeasts, and fungi; e.g., *Acinetobacter*, *Arthrobacter*, *Mycobacteria*, *Actinomycetes*, and *Pseudomonas* among bacteria; *Cladosporium* and *Scolecobasidium* among yeasts |
| **II. pesticides/herbicides** | |
| cyclodiene type (e.g., aldrin, dieldrin) | *Zylerion xylestrix* (fungus) |
| organophosphorus type (e.g., parathion, malathion) | *Pseudomonas* |
| 2,4-D | *Pseudomonas, Arthrobacter* |
| DDT | *Penicillium* (fungus) |
| kepone | *Pseudomonas* |
| piperonylic acid | *Pseudomonas* |
| **III. other chemicals** | |
| bis(2-ethylhexyl)phthalate | *Serratia marascens* (bacteria) |
| dimethylnitrosamine | photosynthetic bacteria |
| ethylbenzene | *Nocardia tartaricans* (bacteria) |
| pentachlorophenol | *Pseudomonas* |
| **IV. lignocellulosic wastes** | |
| municipal sewage | *Pseudomonas*<br>*Thermonospora* (a thermophilic bacterium) |
| pulp mill lignins (various phenols) | yeasts: *Aspergillus*<br>*Trichosporon*<br>bacteria: *Arthrobacter*<br>*Chromobacter*<br>*Pseudomonas*<br>*Xanthomonas* |

Figure 4-11

Degradation pathways of several phenolic compounds by Pseudomonas putida


H$_3$C
OH
OH
O$_2$
CH$_3$
H$_3$C
CHO
COOH
OH
C=O
+H$_2$O
HCOOH
CH$_3$COOH
COOH
CO$_2$
CH$_3$CH$_2$CHOHCH$_2$COCOOH
CH$_3$CH$_2$CHO+
CH$_3$COCOOH
CH$_3$CHOHCH$_2$COCOOH
CH$_3$CHO+
CH$_3$COCOOH

Source: Bull, A.T., etal. (1979)

herbicide, 2,4-D. Likewise, SRI International has undertaken a program to compile a list of common toxic chemicals that are amenable to microbial biodegradation and to isolate and engineer improved strains that might have commercial value.

In general, chlorinated organics are more recalcitrant to biodegradation than are non-chlorinated substances. Thus, persistent pollutants such as DDT, PCBs, etc., represent a more serious challenge to pollution control engineers who are hopeful of applying biological treatment systems to waste management. Microbes exist that can perform chemical transformations of these recalcitrant substances (see Table 4-10), but microorganisms have not yet been isolated that can utilize these compounds as carbon or energy sources. Indeed, it is this lack of direct metabolism by microbes that explains the environmental persistence of compounds such as these. Future success in developing biodegradative systems for pollutants of this type may depend on locating microbial communities consisting of several species of microorganisms which function cooperatively to decompose recalcitrant compounds.

#### 4.5.1.2 Denitrification and desulfurization

The various oxidized forms of nitrogen ($NO_x$) and sulfur ($SO_x$) present serious environmental concerns owing to the ease with which they are converted to strong acids (e.g., nitric and sulfuric) upon exposure to water. The acidiciation of lakes and gound water poses a serious threat to the maintenance of aquatic life and fresh water supplies. Although large amounts of nitrogen (and lesser quantities of sulfur) are nutritional requirements for life, the large-scale burning of sulfur and nitrogen-containing fossil fuels and the release of certain industrial wastes have loaded the environment with toxic levels of these inorganic substances. Traditional schemes for reducing the emissions of these pollutants have been largely physical/chemical in design. Biological processes are under

## Table 4-10

### Type reactions for transformation of chemicals of environmental importance

| Reaction type | Reaction† | Example |
|---|---|---|
| **dehalogenation** | $RCH_2Cl \rightarrow RCH_2OH$ | Propachlor |
| | $ArCl \rightarrow ArOH$ | Nitrofen |
| | $ArF \rightarrow ArOH$ | Flamprop-methyl |
| | $ArCl \rightarrow ArH$ | pentachlorophenol |
| | $Ar_2CHCH_2Cl \rightarrow Ar_2C{=}CH_2$ | DDT |
| | $Ar_2CHCHCl_2 \rightarrow Ar_2C{=}CHCl$ | DDT |
| | $Ar_2CHCCl_3 \rightarrow Ar_2CHCHCl_2$ | DDT |
| | $Ar_2CHCCl_3 \rightarrow Ar_2C{=}CCl_2$ | DDT |
| | $RCCl_3 \rightarrow RCOOH$ | N-Serve, DDT |
| | $HetCl \rightarrow HetOH$ | Cyanazine |
| **deamination** | $ArNH_2 \rightarrow ArOH$ | Fluchloralin |
| **decarboxylation** | $ArCOOH \rightarrow ArH$ | Bifenox |
| | $Ar_2CHCOOH \rightarrow Ar_2CH_2$ | DDT |
| | $RCH(CH_3)COOH \rightarrow RCH_2CH_3$ | Dichlorfop-methyl |
| | $ArN(R)COOH \rightarrow ArN(R)H$ | DDOD |
| **methyl oxidation** | $RCH_3 \rightarrow RCH_2OH$ | Bromacil |
| | $RCH_3 \rightarrow RCHO$ | diisopropylnaphthalene |
| | $RCH_3 \rightarrow RCOOH$ | pentachlorobenzol |
| **hydroxylation** | $ArH \rightarrow ArOH$ | Benthiocarb, Dicamba |
| | $RCH_2R' \rightarrow RCH(OH)R'$ | Carbofuran, DDT |
| | $R(R')CHR'' \rightarrow R(R')CHOH(R'')$ | Bux insecticide |
| | $R(R')(R'')CCH_3 \rightarrow R(R')(R'')CCH_2OH$ | Denmert |
| **β-oxidation** | $ArO(CH_2)_nCH_2CH_2COOH \rightarrow$<br>$ArO(CH_2)_nCOOH + CH_3COOH$ | ω-(2,4-dichlorophenoxy)-alkanoic acids |
| **epoxidation** | $RCH{=}CHR' \rightarrow RCH(O)CHR'$ | Heptachlor |

## Table 4-10 (cont.)

| Reaction type | Reaction† | Example |
|---|---|---|
| **N-oxidation** | $R(R')NR'' \rightarrow R(R')N(O)R''$ | Tridemorph |
| **S-oxidation** | $RSR' \rightarrow RS(O)R'$ or $RS(O_2)R'$ | Aldicarb |
| **=S to =O** | $(AlkO)_2P(S)R \rightarrow (AlkO)_2P(O)R$ | Parathion |
|  | $RC(S)R' \rightarrow RC(O)R'$ | ethylenethiourea |
| **sulfoxide reduction** | $RS(O)R' \rightarrow RSR'$ | Phorate |
| **triple bond reduction** | $RC{\equiv}CH \rightarrow RCH{=}CH_2$ | Buturon |
| **double bond reduction** | $Ar_2C{-}CH_2 \rightarrow Ar_2CHCH_3$ | DDT |
|  | $Ar_2C{=}CHCl \rightarrow Ar_2CHCH_2Cl$ | DDT |
| **double bond hydration** | $Ar_2C{=}CH_2 \rightarrow Ar_2CHCH_2OH$ | DDT |
| **nitro metabolism** | $RNO_2 \rightarrow ROH$ | Nitrofen |
|  | $RNO_2 \rightarrow RNH_2$ | Sumithion |
| **oxime metabolism** | $RCH{=}NOH \rightarrow RC{\equiv}N \rightarrow RC(O)NH_2$ or $RCOOH$ | Aldicarb, Bromoxynil, Dichlobenil |

†Abbreviations: R = organic moiety
Ar = aromatic
Alk = alkyl
Het = heterocycle

Source: Alexander, M. (1981) Science, 211:134.

investigation, however, and several systems have demonstrated feasibility in laboratory-scale applications.

The biological nitrogen cycle entails three phases involving various oxidation states of nitrogen (see Figure 4-12). Atmospheric nitrogen ($N_2$) is relatively inert chemically and must be "fixed" into usable forms such as nitrate and nitrite (oxidized nitrogen) or ammonia (reduced nitrogen). Meanwhile, fixed nitrogen is recycled back into the atmosphere by anaerobic processes. All these steps are carried out by various species of bacteria, according to the chemical reactions shown in Figure 4-12. Of these three phases, the third (denitrification) is the least understood, but it is this process that promises to alleviate pollution problems stemming from excess nitrate and ammonia.

Pollution by sulfur-containing compounds presents a more serious problem than pollution by nitrogenous substances because of sulfur's greater toxicity to living organisms and its greater prevalence in fossil fuels and industrial waste streams. Inorganic sulfur compounds, such as sulfate ($SO_4^=$) and hydrogen sulfide ($H_2S$), can be metabolized by certain microbial species, as shown in the following reactions:

$$SO_4^= \xrightarrow{I} H_2S \xrightarrow{II} S^o$$

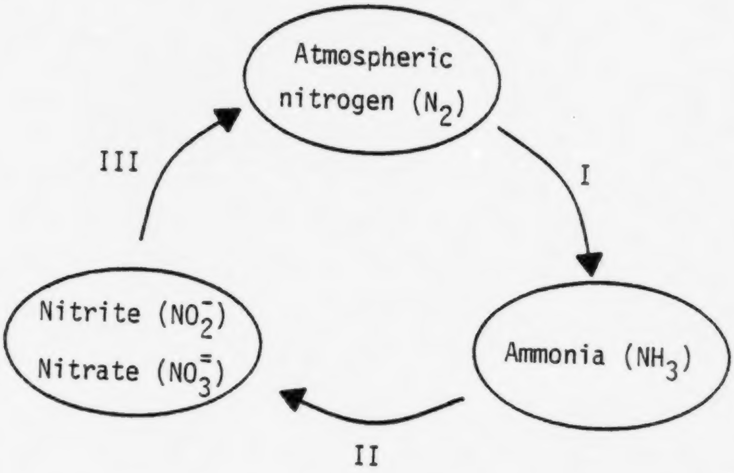
I. *Desulfovibrio desulfuricans*
II. *Chlorobium thiosulfatophilum* or *Chromatium vinosum*

Laboratory-scale systems utilizing these microbial populations arranged serially are under investigation for potential use in treating high-sulfur effluents, such as those arising from coal and gypsum mining and general metallurgical operations.

Fossil fuels may contain up to 7% sulfur by weight. This sulfur is generally in one of three forms:

Figure 4-12

Microbial components of the biological nitrogen cycle


Atmospheric nitrogen ($N_2$)
I
Ammonia ($NH_3$)
II
Nitrite ($NO_2^-$)
Nitrate ($NO_3^=$)
III

I. Nitrogen fixation

*Rhizobium:* $N_2 + H^+ \longrightarrow NH_3$

II. Nitrification

*Nitrosomonas:* $NH_3 + O_2 \longrightarrow NO_2^-$

*Nitrobacter:* $NO_2^- + O_2 \longrightarrow NO_3^=$

III. Denitrification

*Pseudomonas:* $NH_3 + NO_3^= \longrightarrow N_2O$ or $N_2$

- **Organic sulfur,** in which sulfur is covalently linked to carbon either directly as R-S-S-R or R-S-R, or bound as a sulfate, $R-O-SO_3$;
- **Pyritic sulfur** in the form of iron pyrite, $FeS_2$; and,
- **Inorganic sulfate,** $SO_4^=$.

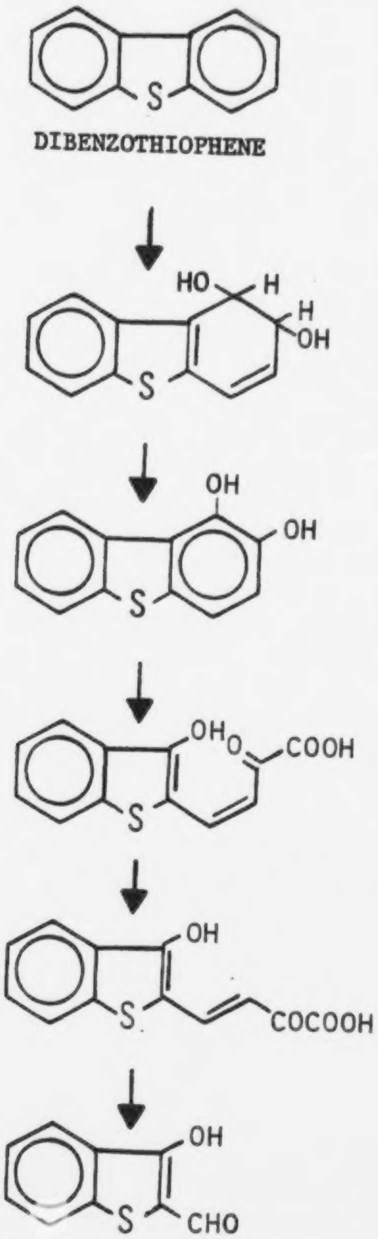
Of these, organic sulfur predominates in crude petroleum, whereas pyritic sulfur and sulfate are found largely in coal. In all cases, the combustion of untreated crude oil or coal releases to the atmosphere huge quantities of sulfur dioxide gas ($SO_2$) and particulate sulfates. These sulfur compounds are intrinsically toxic and, moreover, combine with water to form sulfuric acid. The removal of sulfur compounds from fossil fuels **prior** to combustion has been deemed a desirable adjunct to, or possible replacement for, costly scrubbers now widely used to control stack emissions.

Biological desulfurization is still in the experimental stage, but several microbial systems are under investigation. Pyritic sulfur can be leached from mined coal using Thiobacillus ferrooxidans and Thiobacillus thiooxidans--the same bacterial species employed for mineral leaching in the mining industry. Also, a thermophilic microbe, Sufolobus acidocalderius, has been isolated. All these organisms operate under acidic conditions (pH 1 to 3) and convert sulfides to sulfuric acid. Thus, the pyritic sulfur content of the fossil fuel is transformed into a water soluble compound that can be readily washed away. However, the acid that is generated represents a pollutant in its own right that must be dealt with.

Organic sulfur exists in crude petroleum largely as linear mercaptans (R-SH) or as aromatic thiophenes. Microbial systems for converting thiophenes into water soluble compounds are under development. The biochemistry involved in this transformation is shown in Figure 4-13. The principal drawback of this process lies in the loss of carbon atoms (and, therefore, of Btu content) resulting from the removal of sulfur-containing organics.

Figure 4-13

Pathway of microbial conversion of dibenzothiophene into water soluble compounds


S
DIBENZOTHIOPHENE
HO
H
H
OH
S
OH
OH
S
OH
O
COOH
S
OH
S
COCOOH
OH
S
CHO

### 4.5.1.3 Toxic metals

Biological concentration of heavy metal ions from a dilute waste stream involves processes essentially identical to those described for the mining industry in Section 4.4.1. The emphasis for pollution control, of course, is isolation and **disposal** of toxic metals, whereas ultimate **recovery** of the metals is of concern to the mining industry. The incorporation of metals from an industrial effluent into biological sediments (i.e., activated sludge) has proven to be a satisfactory application of biotechnology to pollution control. The immobilization of metals by these sediments may be the result of (1) direct intracellular uptake, (2) adsorption to cell surfaces, or (3) sequestration in a microbially produced exopolysaccharide matrix. In addition to bacteria, other organisms are used to concentrate metals from dilute waste streams. Settling ponds containing photosynthetic algae or rapidly growing aquatic vegetation, such as water hyacinths, are also fulfilling this purpose.

### 4.5.2 Future prospects

The greatest R&D effort involving near-term applications of biotechnology to pollution control will be in developing improved microbial strains for decontamination of polluted waste waters and for in situ detoxification of contaminated soils and sediments. There exist considerable gaps in our basic knowledge of the types of microorganisms capable of degrading toxic chemicals. In particular, anaerobic bacteria and filamentous fungi represent two diverse classes of microbe for which considerable potential exists for biological pollution control, but little is known of their general properties.

Bacteria are classified into several groups based on the effect that oxygen has on their growth and metabolism:

- **Obligate aerobes** require oxygen for growth. An example is the tubercle bacillus, the causative agent of tuberculosis.

- **Obligate anaerobes** survive only in the absence of oxygen. Examples include clostridia (various species of which cause botulism, tetanus, and gangrene), bacteroides (intestinal bacteria that ferment glucose to form organic acids; e.g., formic, acetic, propionic, butyric, lactic, and succinic), denitrifiers that reduce nitrate to nitrogen gas, sulfate reducers that produce hydrogen disulfide (a source of pollution in anoxic ponds and streams), and methane producers that form marsh gas.

- **Facultative** organsims, such as many enteric bacteria (e.g., E. coli), can thrive with or without oxygen by shifting to different metabolic processes in each case.

Anaerobic bacteria are are particularly relevant to pollution control practices because of their prevalence in sub-soil. Thus, bacteria of this type will encounter toxic chemicals or petroleum wastes that have been spilled, as well as herbicides and insecticides that have been applied to the ground. A subgroup of anaerobic bacteria, called **microaerophilic**, can tolerate or even prefer low oxygen pressures (but much less than in air). These conditions prevail just beneath the surface of the soil. Thus, microaerophiles, about which very little basic information is known, should receive considerable attention for possible future use as in situ decontaminating agents. Likewise, anaerobic bacteria that thrive in underwater sediments, such as anoxic settling ponds or in the bottom of the kepone-laden James River, will be the subjects of more intense research in the years ahead.

Fungi are classified into three groups: (l) single-celled **yeasts**, (2) multicellular filamentous colonies, or **molds**, and (3) **mushrooms.** The filamentous fungi include some well known types, such as Neursopora, Penicillium, and Aspergillus, as well as lesser known aquatic watermolds and soil fungi. The genetics and biochemistry of fungi are much less well understood than are bacteria. However, it is certain that, like bacteria, fungi serve crucial roles in recycling organic matter throughout the biosphere. The contribution made by fungi to the decontamination of polluted soils and streams is becoming better appreciated, and research into the application of fungi to waste management should receive greater attention in the years ahead.

The following list outlines some aspects of applied genetics and waste management that will be under development.

- Cataloging the types of chemical transformations performed by microorganisms and the microbes involved.
- Isolating and characterizing the genetic material and enzymes responsible for the observed transforming activity.
- Conducting genetic engineering on organisms that occur naturally in a particular environment (e.g., river bed sediment) to confer the ability to degrade a pollutant that is **not** normally present in that environment (e.g., kepone). Successful decontamination of polluted sites by in situ biotreatment requires that the engineered microbe will compete favorably with existing microflora.
- Developing biotreatment systems for dealing in situ with specific wastes under a given set of conditions. For example, a chemical spill at a particular site may require a different microbe depending on the ambient temperature, or on the presence of certain nutrients. Exogenous nutrients such as glucose may have to be supplied.
- Designing bioreactors for on-line waste stream treatment. Systems for immobilizing microbes are under develoment. Monitoring and controlling the concentration of toxic substances in the waste stream are vital since excessive doses of most pollutants are deadly even to microbes that thrive on **low** concentrations of these chemicals. Thus, the design and engineering of systems for diluting concentrated wastes prior to biotreatment may be a greater technical challenge than is the development of microbial populations capable of performing the biodegradation.

### 4.5.3 Potential hazards

Biological processes are currently in wide use throughout the pollution control industry, but so far modern applied genetics or genetic engineering has had negligible impact. The potential exists, however, that these new biotechnologies will drastically alter or replace conventional physical/chemical waste management processes. Nevertheless, considerable basic information regarding the relevant biological systems must be acquired before genetic engineering can be implemented to improve on naturally occurring organisms. Very little is now known of the biochemistry, metabolism, genetics, or natural ecology of the microbial species that mediate biodegradative processes. Indeed, the mere identification of potentially useful microorganisms is far from complete. Thus, the impact of applied genetics may not be felt in this industry for five years or more.

Nevertheless, increasing utilization of **natural** bioprocesses in pollution control entails certain potential hazards that are noteworthy and that may forewarn of future risks evolving from the application of genetic engineering in this industry. Chief among these concerns is the generation of biological **aerosols.** These are tiny droplets of water or dust particles containing active microbial material. They remain suspended in the atmosphere to be transported by air currents to distances of several miles from their origin. Many industrial processes have the potential to create hazardous aerosols that contain pathogenic microorganisms. Among these are:

- **Agricultural practices.** Stockyards and poultry feedlots generate contaminated dust aerosols that may elicit very serious health problems, such as anthrax. The increasingly common practice of applying partially treated or untreated municipal sewage to crop lands has led to improved crop yields and has provided an alternative to the direct discharge of sewage into lakes and streams. But this practice gives rise to potentially harmful aerosols and to increased risk of ground water contamination. Waste water from food processing plants has also been utilized in land application programs.

- **Textile mills.** The processing of wool and animal hair produces dust aerosols that are known to contain pathogens, such as the causative agents of anthrax and Q fever.

- **Abattoirs and rendering plants.** The slaughtering and processing of livestock is a serious source of infectious aerosols, occasionally causing epidemics among employees. The condition is exacerbated by livestock farmers or ranchers who frequently rush their stock to market at the first sign of disease among members of the herd.

- **Sewage treatment plants** are probably the most numerous and varied sources of pathogenic aerosols. The bubbling of air through an activated sludge facility and the splashing of sewage water over the rock bed of a trickling filter operation both generate numerous aerosolized particles. Approximately one-half of these droplets are in the size range (1 to 5 microns) that are carried downwind for considerable distances and which are readily inhaled and deposited in the human lung. The magnitude of the potential hazard posed by sewage treatment plants is related to the abundance of these facilities, their proximity to residential areas, the great variety of microbial species found in sewage, and the high frequency of aerosolization from these facilities which are in operation all day throughout the year.

Thus, the threat to public health posed by infectious aerosols is considerable. Moreover, many **laboratory**-associated infections also appear to result from the production of aerosols, rather than from more obvious lab incidents, such as pipetting by mouth, needle and syringe accidents, or simple spills. Clearly, future efforts to minimize risks associated with any microbiological process, including those involving recombinant DNA organisms, should focus on methods of controlling aerosols.

The biodegradation of organic pollutants by indigenous microorganisms is chiefly responsible for the eventual recycling of most environmental wastes. Organic pollutants fall into three general categories:

- **Completely biodegradable,** for which there exist microbes capable of mineralizing the substrate. Examples include relatively simple hydrocarbons and aromatics, such as phenols.
- **Totally recalcitrant,** for which there exist no known microorganisms capable of chemically transforming the substrate, or if so, at a very slow rate. Synthetic plastics, such as polyethylene or polyvinyl chloride, appear to be in this category, as do some polychlorinated aromatic hydrocarbons and pesticides.
- **Co-metabolized** compounds are transformed to some extent by microbes that utilize other substances as sources of energy and biomass. Pollutants in this category, such as DDT, aldrin and heptachlor, are degraded slowly. Microbes have yet to be isolated that can use compounds of this type as nutrients.

The impact of applied genetics in this area will be minimal until more is learned of the types of microorganisms involved. The suitability of naturally occurring microbes for waste cleanup will be examined initially. Particular attention will be paid to in situ decontamination processes. However, several factors mitigate against widespread success in this area.

- The concentration of toxic chemicals at the site of a spill or dump site is often too high to permit survival of any microbe capable of degrading the pollutant.
- Concentrations of toxic chemicals sufficiently low to permit survival (generally less than 1,000 ppm) may be **too** low to sustain bacterial growth. Thus, additional nutrients must be supplied.
- Some toxic compounds, particularly those that are co-metabolized, are partially degraded into substances that are slightly less toxic than the parent compound but which are more readily mobilized (that is, dispersed throughout the local food chain). This is the fate of most polychlorinated organics.

Finally, there exist natural microbial systems capable of concentrating inorganic metal ions from dilute waste streams. This accumulation appears to be associated with chemical transformations of the metal into organic forms. The methylations of mercury and arsenic, for example, are known to occur as a result of microbial action in aquatic sediments. These organic derivatives are more toxic than the corresponding inorganic substances, and they are more readily taken up from the sediments by aquatic animals. Thus, commercial use of microbial systems to remove heavy metal ions from waste waters must be monitored for the release of even more toxic organic derivatives.

# SECTION 5

# SUMMARY AND RECOMMENDATIONS

## 5.1 State of the applied genetics industry

All indications are that the U.S. economy is on the verge of a "biology boom." Excitement over the commercial potential of genetic engineering has been very high, as exemplified by the considerable media attention to this area, as well as the enthusiasm shown by investors. Public expectations are also very high that applied genetics will quickly and effectively solve many societal problems, such as cancer, the energy crisis, our polluted environment, and the world food shortage. The next several years will be crucial to the future development of the biotechnology industry. If few (or none) of the expected benefits from applied genetics are realized in the short run, public enthusiasm for this modern technology may dissipate. Further commercial development will be hampered by a lack of investment capital, and adverse publicity will deter innovative entrepreneurs from entering the field with ideas that could lead to short-term success.

One aspect of applied genetics, recombinant DNA technology, has received the bulk of public attention. These experimental techniques involve the cutting and splicing of genes and the subsequent joining together of DNA from different organisms. This new technology offers the prospect of treating previously incurable genetic diseases, such as sickle cell anemia and hemophilia, and of understanding and eventually curing human cancer. On the other hand, the practice of recombinant DNA has produced the specter of inadvertent creation of new and threatening life forms or of deliberate manipulation of human genes for mischievous purposes. But the risks inherent in recombinant DNA technology are surely much smaller than originally feared (see below). As with other technological advances, recombinant DNA will be applied where it will yield substantial commercial pay-off. Only the pharmaceutical industry is likely to realize near-term returns on investments in this new technology. Other industrial sectors will thoroughly investigate naturally occurring biological systems, including microorganisms and higher plants, for potential commercialization, prior to making significant investments in recombinant DNA technology. Although many non-pharmaceutical firms have initiated in-house programs in recombinant DNA research, it may soon become apparent that this is a case of putting "the cart before the horse." Two reasons for this conclusion are:

- Considerable basic scientific information must be acquired in many areas pertaining to the species of microorganisms and higher plants that will be of commercial interest to non-pharmaceutical firms. For example, the use of genetic engineering to endow microbes with useful characteristics, such as the ability to fix atmospheric nitrogen, salt and drought tolerance, and anaerobiosis, must await a better understanding of the biochemical and genetic basis of these traits.

- Technical advances in recombinant DNA methodology will largely be made in academic laboratories, and they will occur at a faster rate than will commercial developments. Thus, the scientific feasiblity of a particular genetic engineering operation will precede by several years, perhaps, its commercial application. The chief factor contributing to this time lag is the considerable disparity between laboratory and industrial settings for the performance of biological processes. For example, an engineered microbial strain might thoroughly degrade kepone in the laboratory, but be unable to survive in competition with the natural microflora existing in James River sediments. Eventual success in a project of this type requires that more information be gathered regarding indigenous organisms.

Thus, the glamour and attention surrounding recombinant DNA may soon subside in favor of increased interest in naturally occurring organisms. This is particularly likely in non-pharmaceutical industries, although the search for drug-like substances in higher plants and animals promises to reorient the focus in the drug sector as well.

Biotechnologies other than recombinant DNA have received less public attention but, nevertheless, are expected to contribute significantly to the commercial success of the "biology business." Modern fermentation technologies will be applied to relevant operations in all industrial sectors, but the extent of their use will obviously depend on the successful generation of new and useful microorganisms that can be grown on a large scale. Likewise, immobilized bioprocesses, such as on-stream bioreactors for waste stream detoxification, will be utilized only to the extent that other biotechnologies generate worthwile organisms or enzymes for the purpose of attachment. Cell fusion techniques will undergo further development as an alternative to recombinant DNA methods for producing genetically altered organisms. But applications of this technology are limited for the most part to the biomedical field (e.g., monoclonal antibodies) and to the agriculture industry (e.g., fusions of plant cell protoplasts to generate hybrids).

### 5.2 Overall assessment of risk

As with public perception of the possible benefits resulting from applied genetics, considerations of potential risks associated with these technologies has focused on recombinant DNA procedures. As mentioned in Section 4.1.3, several workshops have been held during the past four years to review and summarize the status of risk assessment in the recombinant DNA field. In addition, the NIH (through its Recombinant DNA Advisory Committee, the RAC) has prepared a Risk Assessment Plan which will summarize and update annually information relevant to recombinant DNA risk assessment. The most recent update was published in the September 17, 1980, issue of the Federal Register. A principal component of the plan is to analyze risk data pertaining to three general categories of host-vector systems in common use: prokaryotic (e.g., E. coli

K12), lower eukaryotic (e.g., Saccharomyces cerevisiae), and higher eukaryotic (e.g., mammalian cells). The following excerpt summarizes the current understanding of the risks:

> ...despite intensive study by the RAC Subcommittee on Risk Assessment and NIH staff, several conferences and workshops to consider specific issues and several experiments, no risks of recombinant DNA research have been identified that are not inherent in the microbiological and biochemical methodology used in such research. (45 FR 61874)

Thus, in the absence to date of any compelling evidence to the contrary, and despite assiduous efforts to identify any potential hazards, scientists are now convinced that the practice of recombinant DNA techniques poses no health risks over and above those encountered in normal microbiological research.

Since the NIH committee charged with the task of monitoring the field of recombinant DNA has reached the conclusion that there exist no untoward risks in practicing this technology, what will become of the committee itself? Indeed, what is the future of government involvement generally in this area? Several comments can be made:

- During the past year or so, the RAC has divested itself of oversight responsibilities by delegating many of its functions to the local Institutional Biosafety Committees (IBCs). The IBCs are naturally reluctant to take on the added workload in dealing with what are now deemed innocuous safety issues.
- As are other government agencies, the RAC is beginning to examine issues from a cost/benefit standpoint. The RAC recognizes that other areas of potential health concern exist in the biomedical research field. These more conventional hazards, which exceed the threat of recombinant DNA as potential risks, include exposure to pathogenic aerosols, X-rays, radionuclides, and toxic chemicals. Any future role for the RAC (or some other

RAC-like organization) should include consideration of these safety issues as a priority.

- Biotechnologies other than recombinant DNA have so far received little attention with regard to potential hazards. For example, cell fusions involving various cells derived from human tissues may become increasingly popular as a method for obtaining human biologics for drug manufacture. Large-scale application of this procedure entails the speculative risk that pathogenic viruses will be induced and propagated. Relevant government agencies should be advised to monitor the application of **any** biotechnology within their purview.

### 5.3 Recommendations to the EPA

The wide array of industrial uses of applied genetics can be grouped into two categories with respect to environmental issues: (1) those applications in any industrial sector tnat constitute an **adverse** impact on the environment; and (2) bioprocesses designed to assist in the effort to control pollution and constitute a net **positive** impact on the environment. With this general distinction in mind, specific recommendations can be made:

- The commercial applied genetics industry is at a nascent stage of development and, so far, no incidents of environmental concern related to this industry have materialized. Any environmental risks arising from industrial use of applied genetics are speculative. At this time, there exists no compelling reason for the EPA to establish regulations in this area.

- Should environmental hazards emerge in the future, it is probable that they can be handled within the existing regulatory framework. During the past five years, the Office of the General Counsel at the EPA has examined the applicability of existing legislation, particularly TSCA, to commercial recombinant DNA activities. A consensus appears to have been reached that EPA has the authority to regulate commercial uses of this technology, including: (1) requirements for premanufacture review of industrial processes based on recombinant DNA methodologies (Section 5 of TSCA); (2) restriction or prohibition of manufacture, processing, distribution, or use of recombinant DNA if such is deemed hazardous (Section 6); and (3) dealing with imminent hazards involving recombinant DNA (Section 7). Moreover, the discharge of recombinant DNA material into the environment could be regulated under existing statutes within the Clean Air and Water Acts. In summary, no additional legislation would seem to be necessary in order for the EPA to regulate commercial activities involving recombinant DNA.

- The EPA should continue to take an active role in promoting applied research and development of biological waste management processes and techniques. Emphasis should be placed on the **biology** of relevant systems rather than on process engineering and design. A particularly troublesome problem requiring more research is in situ decontamination of chemical wastes. A more tractable problem deserving EPA attention involves the use of on-line bioreactors for treating industrial effluents at the source.
- The EPA should sponsor further investigation into the generation, dispersal, and control of biological **aerosols.**
- To the best of its abilities, the EPA should monitor commercial and scientific developments in the field of applied genetics with the aim of identifying both imminent environmental hazards and areas where this technology might be applied to pollution control operations.

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# GLOSSARY

This list of definitions is intended to help the reader and should not be considered all inclusive.

| | |
|---|---|
| **antigen** | any chemical substance, natural or man-made, that elicits an immune response in animals |
| **aerobe** | organism requiring oxygen |
| **anaerobe** | organism able to live in the absence of oxygen; some anaerobes are "obligate"; i.e., they are killed in the presence of oxygen |
| **bacteriophage** | one of a subgroup of viruses that infect bacteria; consists of a relatively small amount of DNA contained in a protein coat |
| **chloroplast** | a cellular organelle in higher plants; site of photosynthesis |
| **chromosome** | the basic macrostucture of heredity; organization of DNA in cell nuclei containing large numbers of genes |
| **clone** | a collection of cells each having an identical genetic composition |
| **codon** | a triplet of nucleotides on a DNA chain that specifies a particular amino acid or otherwise controls protein synthesis |
| **colicin** | a bacterial toxin, the coding for which is found on a plasmid; some forms are toxic to humans |
| **conjugation** | one-way transfer of DNA between bacteria in cell contact |
| **crown gall** | plant tumor caused by infection with Agrobacterium tumefaciens; genes located in the Ti-plasmid of the Agrobacterium are responsible for tumor induction |
| DNA | deoxyribonucleic acid; the molecular basis of genes; made from the sequential arrangement of four nucleotide building blocks: adenine, cytosine, guanine, and thymine; normal configuration is in double-stranded helical form |

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|---|---|
| **cDNA** | complementary DNA; laboratory-created DNA that is complementary to mRNA extracted from a cell |
| **entomopathogen** | insect pathogen, usually microbial in nature, such as a bacterium, protozoan, or virus |
| **enzyme** | organic catalyst of biochemical reactions in a cell; composed of protein |
| **eukaryote** | an organism composed of cells that are distinguished by the presence of a nucleus and multiple chromosomes; fungi, protozoa, and all differentiated multicellular forms of life are eukaryotic |
| **F factor** | fertility factor; plasmid that specifies gender in bacteria |
| **gene** | a defined length along a chromosome, made of DNA and coding for a protein molecule |
| **gene library** | the result of a shotgun experiment in which each cloned bacterial colony contains different segments of DNA |
| **genome** | all the genes of an organism or individual |
| **HEPA filter** | high efficiency particulate air filter |
| **host-vector system** | in the recombinant DNA field, the particular organism (host) into which the gene is cloned, and the vehicle (vector)--usually a plasmid system--that carries the gene into the host |
| **intron** | an intervening sequence of DNA of unknown function found only in eukaryotic genes; this sequence is not expressed in the transcription to mRNA |
| **lac operon** | an operon in *E. coli* that codes for three enzymes involved in the metabolism of lactose |
| **lambda** | bacteriophage that infects *E. coli*; commonly used as a vector in recombinant DNA research |
| **ligase** | an enzyme that catalyzes the linking of sequential bases in single-stranded DNA |
| **lignocellulose** | complex biopolymer comprising the bulk of woody plants; consists of polysaccharides and polymeric phenols |

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|---|---|
| **lymphocyte** | a type of cell found in the blood, spleen, lymph nodes, etc. of higher animals; one sub-class of lymphocyte manufactures and secretes anitbodies |
| **lysis** | process of cell disintegration; cell bursting |
| **non-conjugable** | refers to bacterial plasmids that cannot be transferred between organisms |
| **nucleotide** | any of a class of compounds consisting of a purine or pyrimidine base, bonded to a ribose or deoxyribose sugar and to a phosphate group; the basic structural units of RNA and DNA |
| **nucleus** | the cell region containing chromosomes and enclosed in a definite membrane; found only in eukaryotic cells |
| **oligonucleotide** | the sequential arrangement of more than one nucleotide |
| **operator** | a region of DNA that controls the expression of adjacent genes by interacting with a repressor protein |
| **operon** | a gene unit consisting of one or more genes and the controls for that unit; the lac operon, for example, is made up of three genes, the operator, and the terminator |
| **opines** | unusual amino acids synthesized by genes located on Ti-plasmids; nopaline and octopine are examples |
| **peptide** | two or more amino acids joined together |
| **phage** | shortened form of the word bacteriophage; bacterial virus |
| **plasmid** | a small circle of double-stranded DNA that exists and replicates autonomously in bacteria; often codes for resistance to antibiotics; may be transferred between bacteria during conjugation |
| **polymerase** | enzyme that catalyzes the assembly of nucleotides into RNA and of deoxynucleotides into DNA |
| **polypeptide** | a molecular chain of many amino acids joined together; synonymous with protein |
| **prokaryote** | cellular organism distinguished by the lack of a defined nucleus and by the presence of a single, naked chromosome; bacteria and blue-green algae are the only major examples |

| | |
|---|---|
| **promoter** | the region on a DNA strand that indicates the place to start the transcription of the gene into RNA |
| **protein** | a sequence of amino acids; the ultimate expression of a gene; primary component of enzymes and many hormones |
| **protoplast** | a bacterium or plant cell from which the cell wall has been removed |
| **replication** | the process of making copies of DNA in a cell; depending upon the plasmid, man copies may be replicated after insertion of DNA into the host |
| **repressor** | a gene product that prevents the transcription of an operon by binding to an operator region |
| **restriction endonuclease** | one of a class of enzymes that cleave both strands of DNA at sequence-specific sites; used extensively in recombinant DNA experiments |
| **R factor** | resistance factor; refers to plasmids coding for resistance to antibiotics |
| **ribosome** | a large molecular array, composed of RNA and protein, that is responsible for translating messenger RNA into protein |
| **RNA** | ribonucleic acid, used to form complements to DNA in gene expression |
| **mRNA** | messenger RNA, formed in the cell nucleus in the process of gene expression; complementary to the base sequence of the DNA of the gene |
| **shotgun cloning** | cloning procedure in which all the chromosomes of a donor organism are enzymatically fragmented and placed into hosts for expression; results in a gene library |
| **sticky end** | refers to double-stranded DNA that has been cleaved in such a way by certain restriction endonuclease enzymes that the end of one strand extends beyond the end of the other; the end is called "sticky" because the bases are exposed and can thus mate with complementary sticky ends |
| **T-DNA** | a region of the Ti-plasmid that contains genes required for crown gall tumor induction and maintenance |

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|---|---|
| **terminator** | a region on a gene that codes for the termination of transcription |
| **Ti-plasmid** | a large plasmid found in *Agrobacterium tumefaciens*; induces crown gall tumors in plants infected with the bacterium |
| **transcription** | the process of copying DNA into RNA; the result is messenger RNA |
| **transduction** | the transfer of genetic material from one cell to another by means of a viral vector (for bacteria, the vector is bacteriophage) |
| **transformation** | the process of inserting into the host organism a vector containing a gene that is to be cloned |
| **translation** | the process of making a peptide from mRNA; performed by ribosomes |
| **transposons** | short DNA segments containing one or a few genes that are readily translocated between cells or to different sites within the same cell; responsible for antibiotic resistance in bacteria; also found in some eukaryotic organisms; transposons may serve as suitable vectors for genetic engineering in various organisms |
| **vector** | an agent consisting of a DNA molecule known to autonomously replicate in a cell to which another DNA segment may be attached experimentally to bring about the replication of the attached segment |
| **virus** | any of the submicroscopic infective agents composed of RNA or DNA wrapped in a protein coat and capable of growth and multiplication only in living cells |